olume 49 AUGUST, 1951 Number 2

ENDOCRINOLOGY

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SSUED MONTHLY FOR THE ASSOCIATION FOR THE STUDY OF
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ENDOCRINOLOGY

VOLUME 49 AUGUST, 1951 NUMBER 2

NITROGEN-SPARING EFFECT OF CARBOHYDRATE RELATED TO TIME FACTOR WITH HYPOPHYSECTOMIZED AND DIABETIC RATS*

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LARSON and Chaikoff (1937) working with adult dogs, and Cuthbertson, McCutcheon and Munro (1940), and Munro (1949), studying adult humans and rats, showed that the nitrogen-sparing effect of single carbohydrate feedings was dependent upon the time which elapsed between the feeding of the protein and the carbohydrate moiety of the diet. These authors found that no nitrogen-sparing effect could be demonstrated if carbohydrate was fed more than 4 hours before or after the protein meal. It was also shown that the growth of infantile rats (Geiger 1948) and the repletion of protein depleted adult rats (Geiger, *et al.*, 1950) are likewise affected by the temporal separation of feeding protein from the remainder of the diet.

As to the mechanism of the nitrogen-sparing effect of simultaneously fed carbohydrate, no acceptable explanation has yet been offered. The possibility that the caloric-sparing action of the carbohydrate is responsible for the increased nitrogen retention has been disproved by Munro (1949). These experiments, as well as those reported by Geiger, Bancroft and Hagerty (1950) show also that the presence of carbohydrate does not improve intestinal absorption of the protein. The simultaneous feeding of carbohydrate does not seem to facilitate the utilization of protein by providing the necessary precursors of some missing nonessential amino acids as has been discussed by Geiger (1950) and Geiger, Wick and Bancroft (1950). It had to be considered as a further possibility that dietary sugar

Received for publication March 12, 1951.

* Supported by a grant from the Williams-Waterman Fund of the Research Corporation.

may mobilize insulin, which affects nitrogen-retention. Mirsky (1938), McKay, Barnes and Bergman (1939) and Lotspeich (1949) have demonstrated the nitrogen-sparing effect of extra insulin. In the following experiments we therefore investigated whether the nitrogen-sparing effect of sugar fed simultaneously with protein can be demonstrated in depancreatized and alloxan diabetic rats, in which the source of insulin has been removed.

Mirsky (1939) and Gaebler and Robinson (1942) reported on the nitrogen-sparing effect of anterior pituitary extracts, and recently Jordan, *et al.* (1950) reported that sugar-induced eosinopenia is effected through increased activity of the anterior pituitary. In view of these experiments, we extended our investigations to hypophysectomized rats.

TABLE 1. COMPOSITION OF DIETS

Ingredients	Diet No.			
	1	2	3	4
	Gms. fed in Periods I & III		Gms. fed in Period II	
Basal diet[1]	2	—	2	—
Butter	2.8	—	2.8	—
Glucose	4	—	—	4
Casein	—	2.5	—	2.5
$NaHCO_3$	—	0.15	—	0.15

[1] With the following compositions: cornstarch 51.6%, Woodflock (finely powdered wood of low resin content which has been extracted with water and solvents) 14.4%, cod liver oil (U.S.P., Nutritional Biochem. Corp., Cleveland) 14.6%, wheat germ oil (Viobin Corp.) 4%, U.S.P. salt mixture II 15.4%, Ca-pantothenate 5 mg.%, inositol 25 mg.%, choline chloride 250 mg.%, niacin 2.5 mg.%, thiamine 1.2 mg.%, riboflavin 1.5 mg.%, pyridoxine 1.2 mg.%, biotin 0.2 mg.%, and folic acid 0.3 mg.%.

METHODS

Adult male rats of the Sprague-Dawley strain were used. Each rat was placed on wire screen in an individual round cage with funnel-shaped bottom. Urine and feces were separately collected before the morning feeding. Urine was stored with addition of a few drops of sulphuric acid and toluene. The nitrogen content of the pooled samples, usually covering a period of 4 days, was determined according to Kjeldahl. The sugar was determined by the Shaffer-Hartman method as modified by Shaffer and Somogyi (1933). The pancreatectomy was performed according to the method of Pauls and Drury (1942). Alloxan diabetes was induced by the intravenous injection of 50 mg. alloxan per kg. of body weight. The hypophysectomized rats were operated on about 10 days prior to the start of the feeding experiments. The composition of the diet is shown in Table 1. The morning diets, No. 1 and 3 were offered to the rats at 9:00 A.M. and removed at 12 noon. The afternoon diets, No. 2 and 4, were offered at 4:00 P.M. The afternoon diets contained 2.5 gm. of casein mixed to a paste by the addition of .15 gm. $NaHCO_3$ and some water, and was usually eagerly consumed within 1 or 2 hours. The sugar as a "mobile constituent" of the diet was mixed either with the morning diet No. 1 or with the afternoon diet No. 4; i.e., it was fed either separately or simultaneously with protein. For 3 to 6 days before the start of the experiments, the rats were fed the diets used in Period I in order to habituate them to environment and feeding time.

EXPERIMENTAL

A. *Normal Rats*

The data of the experiments on normal, control rats are condensed in Table 2. We tabulate as an example the results of the nitrogen-balance experiment for rat No. 1 *in extenso*. Because the food intake and the fecal nitrogen-excretion during the different periods was practically constant, we report for the further experiments only the values of the urinary nitrogen-excretion. Rats Nos. 1 and 2 demonstrate in confirmation of the reports quoted above that during Period II, when sugar and protein were fed simultaneously, the nitrogen-excretion is decreased. These results were

TABLE 2. NITROGEN INTAKE AND EXCRETION IN NORMAL AND HYPOPHYSECTOMIZED RATS DURING SEPARATE AND SIMULTANEOUS FEEDING OF PROTEIN AND CARBOHYDRATE

Rat No.	Body wt. (g)		Nitrogen	Period I	Period II	Period III	
	Start	End		Separate feeding	Simultaneous feeding	Separate feeding	
1	185	205	Intake (mg.)	1358	1368	1365	Normal
			Feces	130	147	165	
			Urine	885	730	940	
			Balance	+343	+491	+260	
2	195	206	Urine	1176	1014	1134	Normal
3	123	140	Urine	1020	625	780	Hypophysectomized
4	131	143	Urine	1140	770	1250	Hypophysectomized
5	133	140	Urine	907	762	945	Hypophysectomized
6	124	128	Urine	965	685	784	Hypophysectomized
7	136	145	Urine	765	642	700	Hypophysectomized

further supported by experiments on eight normal rats and on twelve protein-depleted rats (Geiger, Bancroft, Hagerty), which all demonstrated that simultaneous feeding of protein and sugar in Period 2 promotes nitrogen retention.

In the control experiments, where in all three periods only protein has been fed and in six experiments where, instead of sugar, fat has been fed simultaneously with protein, we found, in confirmation of Munro, that the nitrogen retention in the second period has not been altered in comparison with Periods 1 and 3.

B. *Hypophysectomized Rats*

The results of the experiments on hypophysectomized rats are tabulated in Table 2, Rats Nos. 3–7. The fecal-nitrogen excretion during the different periods was practically constant; hence only urinary nitrogen-excretion is reported. Each period comprises 3 consecutive days. The food consump-

tion was in all 3 periods constant. Like the normal rats, the hypophysectomized animals excreted less nitrogen during Period II, when sugar and protein were fed simultaneously, than during the control Periods I and III when they were fed separately.

C. *Diabetic Rats*

The experiments on depancreatized and alloxan diabetic rats have been treated as a single group. The experimental data are condensed in Table 3.

TABLE 3. THE SUGAR AND NITROGEN EXCRETION OF DIABETIC RATS DURING THE SEPARATE AND SIMULTANEOUS FEEDING OF PROTEIN AND CARBOHYDRATE, WITH AND WITHOUT INSULIN

Rat No.	Type of diabetes	Units of insulin daily	Urine excretion	Period I	Period II	Period III
				Separate feeding	Simultaneous feeding	Separate feeding
1	Alloxan	0	Vol. ml.	40	32	28
			Sugar mg.	6216	1716	900
			N. mg.	1460	881	960
2	Alloxan	1	Vol. ml.	21	31	26
			Sugar mg.	112	2008	400
			N. mg.	910	646	975
		0	Vol. ml.	48	134	55
			Sugar mg.	3372	10710	4250
			N. mg.	1090	830	910
3	Alloxan	1	Vol. ml.	58	41	33
			Sugar mg.	592	1476	464
			N. mg.	992	760	932
		0	Vol. ml.	89	174	160
			Sugar mg.	7000	12760	12330
			N. mg.	936	877	906
4	Pancreas	1	Vol. ml.	54	19	26
			Sugar mg.	861	541	180
			N. mg.	840	672	816
5	Pancreas	1	Vol. ml.	57	31	80
			Sugar mg.	1512	1496	4360
			N. mg.	1240	890	1063
		0	Vol. ml.	136	163	140
			Sugar mg.	10240	13440	11840
			N. mg.	980	943	963

The animals in these experiments consumed in the 3 periods equal amounts of food, and the dietary intakes were similar to those of the control animals. The diabetic rats excreted large amounts of urine and sugar in the experiments in which no insulin was supplied. Hence, their insulin production was severely deficient. It was considered that sugar might have to be metabolized in order to increase the retention of simultaneously fed nitrogen. Therefore, each rat was given 0.5 unit of protamine-zinc insulin

(Lilly) injected subcutaneously twice daily, at 9 A.M. and 12 noon. We thus maintained a minimum supply of insulin to insure some metabolism of sugar.

Table 3 shows that in all except one experiment the urinary nitrogen-excretion was decreased in Period II. In this period sugar and protein were fed simultaneously. The urine-volume and sugar excretion did not show any regular changes in the different periods. The nitrogen-sparing effect of simultaneously fed sugar was of the same order as in normal animals. Since the islands of Langerhans were severely damaged in the animals of this group, an extra insulin production, elicited by the alimentary sugar-load, does not appear to be the reason for the nitrogen-sparing effect.

SUMMARY

The nitrogen-sparing effect of sugar fed simultaneously with protein has been investigated with diabetic and hypophysectomized rats. Like normal animals, these rats excreted less nitrogen during the period of simultaneous feeding than when the sugar and the protein were fed separately. The results suggest therefore that the Langerhans Islands and the pituitary are not involved in the nitrogen-sparing action of sugar fed simultaneously with protein.

REFERENCES

CUTHBERTSON, D. P., A. MCCUTCHEON AND H. N. MUNRO: *Biochem. J.* **34:** 1002. 1940.
GAEBLER, O. H. AND A. R. ROBINSON: *Endocrinology* **30:** 629. 1942.
GEIGER, E.: *Science* **108:** 42. 1948.
GEIGER, E.: *Science* **111:** 594. 1950.
GEIGER, E., R. W. BANCROFT AND E. B. HAGERTY: *J. Nutrition* **42:** 577. 1950.
GEIGER, E., A. N. WICK AND R. W. BANCROFT: *18th International Physiol. Congress,* Copenhagen. 1950.
JORDON, P. H., G. H. LAST, J. PITESKY AND E. BOND: *Proc. Soc. Exp. Biol. and Med.* **73:** 243. 1950.
LARSON, R. S. AND I. L. CHAIKOFF: *J. Nutrition* **13:** 287. 1937.
LOTSPEICH, W. D.: *J. Biol. Chem.* **179:** 175. 1949.
MCKAY, E. M., R. H. BARNES AND H. C. BERGMAN: *Am. J. Physiol.* **126:** 155. 1939.
MIRSKY, I. A.: *Am. J. Physiol.* **124:** 569. 1938.
MIRSKY, I. A.: *Endocrinology* **25:** 52. 1939.
MUNRO, H. N.: *J. Nutrition* **39:** 375. 1949.
PAULS, F. AND D. R. DRURY: *Am. J. Physiol.* **137:** 242. 1942.
SHAFFER, P. A. AND M. SOMOGYI: *J. Biol. Chem.* **100:** 695. 1933.

BIOLOGICAL ACTIVITY OF STEROIDS AS DETERMINED BY ASSAY OF RENIN-SUBSTRATE (HYPERTENSINOGEN)

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BILATERAL adrenalectomy of normal rats causes a lowering of the level of plasma renin-substrate (hypertensinogen) (Gaudino, 1944). Treatment of such animals with desoxycorticosterone or with 17-hydroxy-11-dehydrocorticosterone restores renin-substrate to normal or near normal. These findings suggest that the assay of renin-substrate in the plasma of adrenalectomized rats before and after the administration of steroids might provide new information concerning the relative biological activity of these compounds. Therefore, experiments were performed with steroids on normal rats and with ACTH on normal and hypophysectomized rats in the hope that additional information could be obtained regarding the mechanism of renin-substrate formation.

METHODS AND MATERIALS

Assay of Renin-Substrate in Plasma

1 ml. of heparinized plasma was mixed with 3 ml. of angiotonase-free hog renin equivalent to 3 Goldblatt units and incubated for 5 minutes in a water bath at 37° C. At the end of the incubation, dilute HCl was added to adjust the pH to 5.1–5.2; the mixture was then placed in a boiling water bath for 10 minutes. The coagulated protein was removed by centrifugation and the clear supernatant fluid poured off and tested for angiotonin by intravenous injection in pithed cats prepared as described by Shipley, Helmer, and Kohlstaedt (1947). The various samples of supernatant fluid were compared to a lyophilized laboratory standard of angiotonin, 30 micrograms of which is designated as 1 unit. The quantity of renin-substrate found in the plasma is expressed as units of angiotonin formed by 1 ml. of plasma.

Experimental Animals

Male rats of the Long-Evans strain weighing 175 to 350 gm. were used. These included normal, adrenalectomized, and hypophysectomized groups. All animals were fed a stock diet. The adrenalectomized and hypophysectomized animals received 0.9% saline solution as drinking water.

The adrenalectomized animals were prepared under ether anesthesia, both adrenals being removed through a midline abdominal incision. Care was taken not to rupture the adrenal capsule.

Received for publication March 13, 1951.

The hypophysectomized animals were purchased from the Hormone Assay Laboratories, Chicago, Illinois.

Hormonal Preparations

1. ACTH (Adrenocorticotropin) Armour
2. Adrenal Cortex Extract, Upjohn
3. Adrenal steroids (See Table 3)

While Cortisone (17-hydroxy-11-dehydrocorticosterone) was suspended in an aqueous medium, all other steroids were dissolved or suspended in sesame oil.

Initially each steroid was given as a single dose—2 mg. per rat every 24 hours for 7 days. Those that caused an increase in renin-substrate were further studied to determine the minimum amount that would produce the maximum response.

All injections were made subcutaneously. The sites of injection were methodically varied so that any single location would not be used twice. A second compound was not tested in the same animal until at least two weeks had elapsed.

Bleeding Methods

The rats were lightly anesthetized with ether. Then 2 to 3 ml. of blood was obtained by intracardiac puncture with a heparin-moistened syringe to which a clean, sterile 24-gauge needle had been attached. Samples were taken immediately before and after each course of steroid administration.

CONTROL EXPERIMENTS

Renin-Substrate Values in Normal Rats

The renin-substrate was assayed on 72 normal rats. The average value was 4.13 angiotonin units per milliliter of plasma with a range of 2.4 to 6.0 units. In 63 out of the 72 rats the values fell between 3.2 and 5.0 angiotonin units.

Effect of Bleeding on Renin-Substrate

In order to determine whether the amount of blood removed would affect the results of the assays, three normal rats were bled at 0, 1, 3, and

TABLE 1. EFFECT OF BLEEDING ON RENIN-SUBSTRATE*

Rat No.	Time of bleeding in hours				
	0	1	3	24	72
28	4.0			4.0	
29	4.0			4.0	
30	4.0			3.8	
31	3.2	3.0	2.8	3.6	
32	5.0	5.0	3.6	5.0	
33	5.0	5.0	4.0	7.0	
57	4.0				4.25
58	4.0				4.0
59	4.0				3.8
60	4.0				4.25
61	4.0				4.0
62	4.4				3.9

* Expressed as angiotonin units per ml. of plasma.

24 hours, three at 0 and 24 hours, and six at 0 and 72 hours. As shown in Table 1, the substrate level was not significantly changed when blood was drawn at daily intervals or longer.

EFFECT OF ADRENALECTOMY ON RENIN-SUBSTRATE

Renin-substrate was determined in the plasma of two series of rats, one given tap water and the other 0.9% sodium chloride solution as drinking water.

In the series in which the animals received tap water the renin-substrate dropped rapidly; values as low as 0.35 angiotonin units per milliliter of

TABLE 2. EFFECT OF ADRENALECTOMY ON RENIN-SUBSTRATE* OF RATS GIVEN 0.9% SODIUM CHLORIDE AS DRINKING WATER

Rat No.	Control	Days after adrenalectomy					
		10	11	14	28	35	49
28	4.0	1.8					
29	4.0	1.8					
30	3.8	1.2					
34		1.6					
35		1.6					
36		1.7					
37			1.0				
38			1.0				
39			1.5				
40			0.35				
48				2.5	2.2	2.4	1.98
49				2.5	1.8	2.0	1.0
50				2.5	2.0	1.75	1.36
51				1.4	1.1	1.1	0.96
52				2.1	1.55		1.6
53				1.7	1.6	1.5	1.26

* Expressed as angiotonin units per ml. of plasma.

plasma were found on the seventh day after adrenalectomy. The animals, if not treated, died on the 7th–11th day. Due to the rapid decrease in substrate and the poor physiological condition these animals were considered to be of little value for routine testing.

In Table 2 is shown the assay of renin-substrate in the series given 0.9% sodium chloride solution as drinking water. The substrate dropped at a reasonable rate in the majority of the animals and was maintained at one-fourth to one-half the normal value for long periods. Several compounds could be tested on one rat over a period of months.

RESULTS

In Adrenalectomized Rats

The results of the assay of steroids for biological activity are shown in Table 3. Specific activity was calculated on the basis of the greatest increase in renin-substrate per milligram of steroid injected per day over a 7-

TABLE 3. THE EFFECTS OF STEROIDS ON THE RENIN-SUBSTRATE OF ADRENALECTOMIZED RATS

Line no.	Compound	No. rats	Dose mg./ day	Angiotonin units/ml. plasma						Angiotonin units/ mg.	Relative activity DCA =100
				Control period	Days on compound				Change		
					3	6	7	11			
1	DCA Desoxycorticosterone acetate	4	0.05	1.06			1.76		0.70	14.0	
		3	0.10	1.36	1.84				0.48		
		3	0.10	1.12			2.68		1.56	15.6	
		2	0.25	1.00		2.60			1.60	10.4	100
		3	0.50	1.60		2.66			1.06		
		3	2.00	1.63			2.88		1.25		
		4	5.00	1.28			2.90		1.62		
2	Compound A 11-dehydrocorticosterone	4	0.25	1.36	1.89				0.53		40
3	Compound E 17-hydroxy-11-dehydrocorticosterone	4	0.10	1.55			1.58		0.03		
		4	0.25	1.32			1.86		0.54	2.16	
		3	0.50	0.60			2.48		1.88	3.76	24
		4	1.00	1.22			3.10		1.88	1.88	
		3	5.00	0.94			3.78		2.84		
4	21-acetoxy-pregnenolone	4	0.50	1.89			2.12		0.23	0.46	4
		3	2.00	1.11			2.45		1.34	0.67	
5	Adrenal cortex extract (Dose: 0.5 cc. per day)	3		1.40				1.96	0.56		
	(Dose: 1.0 cc. per day)	3		1.86				2.80	0.94		
6	Δ^5-pregnene-3β-21-diol-20-one, diacetate	3	2.00	1.06			1.84		0.78		1.6
7	Compound S 17-ahydroxy-11-desoxycorticosterone-21-acetate	6	2.00	1.36		1.83			0.47	0.24	1.5
8	Δ^1-allopregnene-17α-21-diol-3, 20,dione-21 acetate	4	2.00	1.34			1.74		0.40	0.2	1.3
9	Δ^4-androstene-17α-ol-3-one acetate	3	2.00	1.76			1.93		0.17	0.09	0.6
10	Δ^1-allopregnene-17α-ol-3,20-dione	4	2.00	1.53			1.64		0.11	0.06	0.4
11	3-acetoxy bisnorcholenic acid	3	2.00	1.48			1.57		0.09	0.05	0.3
12	Compound G allopregnane-3, 17,21-triol 11-20 dione	4	1.00	1.15			0.94		−0.21		0
13	Progesterone	4	2.0	1.45			1.37		−0.08		0
14	Δ^5-pregnene-3β-17α-21 triol-20 one, 3,21 diacetate	3	2.0	1.23	1.06				−0.17		0
15	Pregnenolone	4	2.0	1.29			0.82		−0.47		0
16	Δ^5-androstene-3β-ol-17-one	4	2.0	1.11			0.82		−0.29		0
17	3-ethoxy $\Delta^{3,5}$-androstadiene-17-one	4	2.0	1.31			1.13		−0.18		0
18	16-dehydropregnenolone acetate	4	2.0	0.95			0.79		−0.16		0
19	Pregnanedione	3	2.0	1.88			1.14		−0.74		0
20	Pregnanetriol (β)	3	2.0	1.03			0.70		−0.33		0
21	Urandiol-3β-11	2	2.0	2.27			1.95		−0.32		0
22	17α hydroxy progesterone	4	2.0	0.89			0.78		−0.16		0
23	Allopregnane-3β-17α diol-20 one-3 monoacetate	3	2.0	1.55			1.39		−0.16		0
24	Pregnenolone-acetate	3	2.0	1.14			0.84		−0.30		0
25	Allopregnane-3β,17α-diol-20-one	3	2.0	0.80			0.80		0.00		0
26	Δ^{16}-dehydroprogesterone	3	2.0	1.45			1.25		−0.20		0
27	$\Delta^{1,4}$pregnadiene-3,20-dione	4	2.0	2.06			1.50		−0.56		0
28	Allopregnane, 3β,17α,21-triol-20-one-3,21-diacetate	4	2.0	2.07			1.74		−0.33		0
29	Pregnane-3β,17α,21 triol-20-one-3,21-diacetate	2	2.0	1.42			1.22		−0.20		0
30	Allopregnane, 3β,21-diol-20-one, diacetate	2	2.0	2.33			2.16		−0.17		0
31	Pregnane-3β,17α-diol-20 one, 3 acetate	2	2.0	1.31	1.12				−0.19		0
32	16,17, oxido-5-pregnene-3β,20 one-3-acetate	3	2.0	0.51	0.41				−0.10		0
33	Δ^4-pregnene-17α,20(β)1 triol-3 one 20, 21 diacetate	4	2.0	0.81			0.59		−0.22		0
34	17-hydroxy pregnenolone acetate	4	2.0	1.46	1.17				−0.29		0
35	Allopregnane-17α-ol-3,20-dione	2	2.0	1.59			1.21		−0.38		0

day period. Since desoxycorticosterone acetate (DCA) showed the greatest activity of the complunds tested, it was assigned a value of 100, and the others were compared to it. The value of "A" (11-dehydrocorticosterone) was estimated by comparing its activity to the results produced by the administration of 0.1 mg. of DCA in a 3-day assay.

With DCA the maximum increase in renin-substrate was obtained with 0.1 mg. Doses as large as 5 mg. per day did not produce significantly higher substrate concentrations. In contrast, increasing doses of Compound E did tend to cause increased renin-substrate production.

The presence of the 17-OH group definitely reduced the ability of the steroid to increase the renin-substrate as shown by the relative values of DCA and Compound S (lines 1 and 6) and Δ^5-pregnene-3β-21-diol-20-one, diacetate and its 17-OH analogue (lines 6 and 14). Although the data on Compound A is inadequate due to lack of material, this relationship seems to hold here also when compared to Compound E (lines 2 and 3).

Of the compounds assayed only those with the side chain —$COCH_2OH$ at 17 were definitely active.

The steroids with Δ^4 unsaturation were the most active; Δ^5 compounds were very much less active. Compounds with any other arrangement of double bonds had no significant effect on increasing the production of substrate.

In Normal Rats

In Table 4 is shown the assay of renin-substrate in normal rats treated with various steroids and ACTH. A definite rise in renin-substrate followed the daily injection of 2.5 or 5.0 mg. of ACTH once a day for 3 days. Neither Cortisone nor DCA produced a significant elevation in renin-substrate. The inconsistent results with three lots of adrenal cortex extract (Upjohn) cannot be explained. The first lot caused a definite increase in renin-substrate. Controls were run on two occasions with identical results before the administration of the ACE, and two weeks after the ACE was stopped, the renin-substrate had returned to normal. Repeating the assays with two other lots of ACE, one showed a minimum rise in renin-substrate, the other no increase.

TABLE 4. EFFECT OF ACTH AND STEROIDS ON RENIN-SUBSTRATE OF NORMAL RATS

Line No.	Compound	No. of rats	Dose mg./day	Angiotonin units per ml.				
				Control period	Days on compound			Change
					3	7	14	
1	ACTH	3	2.5	3.64	4.18			0.54
2	ACTH	3	5.0	3.56	5.13			1.57
3	ACE (Upjohn) 0.5 cc./day	3		4.00		5.50	5.24	1.50
4	ACE (Upjohn) 0.5 cc./day	5		3.91		4.28		0.37
5	ACE (Upjohn) 1.0 cc./day	6		4.30		3.79		−0.51
6	DCA	8	2.0	3.95		4.06		0.11
7	Cortisone	8	5.0	3.95		4.14		0.19
8	Progesterone	4	2.0	4.48		4.60		0.12
9	Testosterone	4	2.0	4.32		4.27		−0.05

In Hypophysectomized Rats

The average renin-substrate content of the plasma of 14 rats of the Sprague-Dawley strain, hypophysectomized one to two weeks previously, was 2.33 angiotonin units per milliliter. This value is distinctly lower than the average normal value of 4.13 units.

When one dose of 3 mg. of ACTH per day was injected for three days into rats that had been hypophysectomized seven days previously, a rise in renin-substrate comparable to that obtained with ACTH in normal rats was obtained. When ACTH was administered two weeks after hypophysectomy using the same dosage schedule as above, only a minimal increase in renin-substrate was demonstrable. When 5 mg. per day was given in divided doses, a fair response occurred at the end of three days in rats that had been hypophysectomized for two weeks.

DISCUSSION

The determination of renin-substrate in the plasma of adrenalectomized rats after the administration of steroids offers another means of screening the biological activity of these compounds qualitatively and quantitatively.

Renin-substrate is a protein associated with the α_2 globulin fraction of the plasma (Plentl, Page and Davis, 1943). It is probably formed in the liver (Page, McSwain, Knapp, and Andrus, 1941; Leloir, Munoz, Taquini, Braun-Menendez, and Fasciolo, 1942). The results of the present study do not necessarily mean that this test offers a means of measuring the protein-synthesizing action of steroids, particularly since DCA is not considered to have an effect on protein metabolism. The active compounds probably restore the metabolic activity of the liver so that renin-substrate can be synthesized at a near normal rate. It is interesting to note that DCA restores the ability of adrenalectomized rats to regenerate liver protein (Friedgood, Vars, and Zerbe, 1950). Furthermore the relative ability of DCA, 11-dehydrocorticosterone and 17-hydroxy-11-dehydrocorticosterone to restore the liver's regenerative power to form new tissue protein is similar to their activity in raising the renin-substrate of the plasma of adrenalectomized rats.

DCA and Compound E do not affect the renin-substrate of normal rats. These two compounds may suppress the endogenous production of corticoids in the normal animals so that actually an increased quantity of steroids was not present in the animal to raise the rate of renin-substrate production. On the other hand, ACTH caused a definite rise in renin-substrate in both normal and hypophysectomized rats. This confirms in another animal the rise in renin-substrate found by Haynes, Forsham, Thorn, and Dexter (1949) in normal dogs treated with ACTH. Although a

quantitative technique for assaying ACTH preparations could be developed by the method reported in this paper, the relatively large doses necessary (2.5 to 5.0 mg. per day for 3 days) make the method impractical.

It was interesting to note the low renin-substrate values found in hypophysectomized rats. Kohlstaedt, Page and Helmer (1940) reported no appreciable change in renin-substrate in dogs after hypophysectomy. In the light of present knowledge, however, the methods used by these authors were hardly specific for renin-substrate. Houssay and Dexter (1942) state that hypophysectomized dogs responded to renin as well as normal dogs, but they did not report the actual assay of renin-substrate.

Since DCA and Compound A cause an elevation of the renin-substrate of adrenalectomized animals, the test reported in this paper cannot be used for screening compounds for antiarthritic action. However, since Cortisone is quite active in elevating the renin-substrate of the plasma of adrenalectomized rats, it is quite possible that compounds that have little or no ability to elicit this response may be of doubtful value in the treatment of arthritis. In the Arthritis Clinic at the Indianapolis General Hospital no compound that was found to be inactive or had relatively little activity in this test was effective in the treatment of arthritis. The compounds tried were: 21-acetoxypregnenolone; pregnenolone acetate; 16-dehydropregnenolone acetate; Δ'-allopregnene-17α-ol-3,20-dione; allopregnane-3β-17α-diol-20-one-3-monoacetate; Δ^{16}-dehydroprogesterone; 3-ethoxy-$\Delta^{3,5}$-androstadiene-17-one; and Compound S.

SUMMARY

A test for the biological activity of steroids based on the determination of the renin-substrate content of rat plasma is presented.

Of the better known compounds assayed, assigning DCA the value of 100, Compound A = 40, Compound E = 24, 21-acetoxypregnenolene = 4, Δ^5-pregnene-3β-21-diol-20-one, diacetate = 1.6, Compound S = 1.5, Compound G = 0, and pregnenolene = 0.

DCA and Compound E, which will raise the plasma renin-substrate level of adrenalectomized rats to normal or near normal values, do not elevate the renin-substrate of normal rats.

ACTH increases the renin-substrate content of the plasma of normal and hypophysectomized rats. The renin substrate content of the plasma of untreated hypophysectomized rats is lower than normal.

Acknowledgements

The authors wish to thank Dr. I. V. Sollins of Chemical Specialties Inc., New York for supplying many of the compounds, Dr. H. Mason of the Mayo Clinic for samples of Compound A and Compound G, and Dr. J. W. Frost for the data from the Arthritis Clinic of the Indianapolis General Hospital.

REFERENCES

FRIEDGOOD, C. E., H. M. VARS AND J. W. ZERBE: *Am. J. Physiol.* **163:** 354. 1950.
GAUDINO, N. M.: *Rev. Soc. argent. de biol.* **20:** 546. 1944.
HAYNES, F. W., P. H. FORSHAM, G. W. THORN AND L. DEXTER: *Fed. Proc.* **8:** 70. 1949.
HOUSSAY, B. A. AND L. DEXTER: *Ann. Int. Med.* **17:** 451. 1942.
KOHLSTAEDT, K. G., I. H. PAGE AND O. M. HELMER: *Am. Heart J.* **19:** 92. 1940.
LELOIR, L. F., J. M. MUNOZ, A. C. TAQUINI, E. BRAUN-MENENDEZ AND J. C. FASCIOLO: *Rev. argent. de cardiol.* **9:** 269. 1942.
PAGE, I. H., B. MCSWAIN, G. M. KNAPP AND W. D. ANDRUS: *Am. J. Physiol.* **135:** 214. 1941.
PLENTL, A. A., I. H. PAGE AND W. W. DAVIS: *J. Biol. Chem.* **147:** 143. 1943.
SHIPLEY, R. E., O. M. HELMER AND K. G. KOHLSTAEDT: *Am. J. Physiol.* **149:** 708. 1947.

FAILURE OF NERVE-BLOCKING AGENTS TO PREVENT THE EFFECTS OF PLACENTAL GONADOTROPHINS IN THE RABBIT[1]

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INTRODUCTION

THE mechanisms by which human chorionic and equine gonadotrophins (HCG and PMS, respectively) stimulate the ovary are incompletely understood. The literature on this subject has been summarized by Zondek and Sulman (1945) and Evans and Simpson (1950). Both gonadotrophins are much more effective in the normal than in the hypophysectomized animal. It is now generally agreed, following the lead of Leonard (1934), that HCG synergizes with pituitary follicle stimulating hormone (FSH) to activate ovarian hypertrophy, estrogen secretion and follicular maturation and luteinization. The report of Friedman (1932) that intrafollicular injection of HCG induces luteinization in the rabbit ovary and the finding of Hill and Parkes (1931) and Hinsey and Markee (1933), that intravenous HCG stimulates ovulation in the recently hypophysectomized mature rabbit, do not contradict the synergism hypothesis; in either case HCG may have synergized with FSH already liberated from the hypophysis. However, in the normal immature rat both HCG and PMS produce a strikingly greater gonadotrophic reaction than does pituitary luteinizing hormone (LH), which is also synergic with FSH. The inference is that HCG and PMS actually stimulate FSH release from the adenohypophysis, a feat which LH is unable to do.

Even if the placental gonadotrophins do stimulate the hypophysis, an involvement of the nervous system in this activation would appear to be denied by the finding that HCG is effective in the absence of the hypothalamus and higher brain centers (Hinsey and Markee, 1933). Nevertheless, in 1948 Kehl and Molina reported that intravenous injections of procaine (novocaine) blocked the ovulatory effect of PMS in the rabbit. They later (1949a) stated that they had blocked ovulation in response to either PMS or HCG by injecting a few drops of procaine directly into the

Received for publication March 15, 1951.

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hypothalamo-hypophyseal region, a method by which Westman and Jacobsohn (1942) had earlier blocked the coital stimulus. Still more recently Kehl and Molina (1949a) and Molina and Douard (1950) found that not only could the effects of PMS be prevented by the intravenous injection of a wide variety of anesthetic, anticholinergic, and antihistaminic agents but also that intravenous procaine, if injected within a minute post coitum, would block the coital stimulus from inducing ovulation. We have not been able to confirm this finding (Sawyer *et al.*, 1951).

Prior to and coincident with the work of the Algerian group, however, we were able to block neurogenic stimulation of the ovulatory discharge of pituitary gonadotrophin in both the rabbit and the rat with the use of antiadrenergic, anticholinergic and anesthetic agents (Sawyer *et al.*, 1947, 1949, 1950; Everett *et al.*, 1949, 1950). The present experiments were undertaken to determine whether the implications of nervous intermediation in the actions of HCG and PMS could be confirmed by preventing the effects of the placental gonadotrophins with these proven blocking agents as well as the drugs employed by Kehl's group. The results are completely negative. Not only have Dibenamine, SKF-501, atropine, Banthīne and Nembutal failed to affect the ovulatory action of HCG or PMS in the rabbit but so have procaine and Neo-Antergan. SC-1950, a more potent ganglionic blocking agent than tetraethylammonium also failed completely against either gonadotrophin. Our results indicate that, if pituitary stimulation is exerted by placental gonadotrophins, it is a direct action on the hypophysis rather than an indirect mechanism involving nervous mediation.

MATERIALS AND METHODS

Forty-five female rabbits, ranging in weight from 2.0 to 5.0 kg. (average 3.3 kg.) were employed in this study. They were isolated for at least two weeks before treatment and a few were used a second time a month later. All were sexually mature except four (weight, 2.0–2.3 kg.) which were selected to test the factor of immaturity in the response to placental gonadotrophins. Most of the rabbits were anestrous at the time of injection. In a few preliminary experiments such animals were primed with estrogen, but this was soon found to be unnecessary. In a typical experiment 500 I.U. of either HCG or PMS was injected subcutaneously after the "blocking agent," injected previously, should have reached maximal effectiveness. Laparotomy or autopsy was performed 24–72 hours later, and a few ovaries were removed for histological examination. Positive criteria of non-blocked gonadotrophic activity were ruptured ovarian follicles as visualized at laparotomy or histologically ascertained luteinization of hemorrhagic follicles.

We used the same brand of PMS as did Kehl *et al.*, i.e., Schering's "Anteron." Our chorionic gonadotrophin was Ayerst's "A.P.L." Both preparations were dissolved in sterile water containing 0.5% phenol; both were employed at a concentration of 500 I.U. and were stored in a refrigerator at 5° C. Even though refrigerated, the PMS solution was discarded if not used up within a week.

Routine procedures such as making up and administering blocking agents have been described previously (Sawyer *et al.*, 1949, 1950, 1951). Dibenamine (N, N-dibenzyl-β-

chlorethylamine) and SKF-501 (N-[9-Fluorenyl]-N-ethyl-β-chlorethylamine) are potent adrenergic blocking agents (Nickerson, 1949). Atropine sulfate and Banthine (β-diethylaminoethyl xanthene-9-carboxylate methobromide) (Hambourger *et al.*, 1950), are universal anticholinergic agents. Nembutal (pentobarbital sodium) is a widely used central depressant and anesthetic. Procaine, although usually classified as a local anesthetic, has anticholinergic and antihistaminic properties (Dutta, 1949). Neo-Antergan (pyranisamine maleate) is a powerful antihistamine (Reuse, 1948). SC-1950 (2, 6-dimethyl-1, 1-diethyl piperidinium bromide) is a ganglionic blocking agent about 5–7 times as effective as tetraethylammonium (Cook *et al.*, 1950). We have found all but the last three drugs effective in preventing neurogenic stimulation of the release of pituitary gonadotrophin (Sawyer *et al.*, 1950, 1951). Procaine and Neo-Antergan were among the agents employed by Kehl and Molina (1948, 1949), and SC-1950 was selected as a more potent

TABLE 1. ATTEMPTS WITH ANTI-NERVOUS AGENTS TO BLOCK THE EFFECTS OF HUMAN CHORIONIC GONADOTROPHIN (HCG) IN THE RABBIT

Drug	Dosage (mg./kg.) and route	Interval between drug and HCG* injections	Number of rabbits	Average weight kg.	Results	
					Number ovulated	Number blocked by drug
—	—	—	5	3.0	5	—
Dibenamine	30 i.v.	1.5 hrs.	2	3.6	2	0
SKF-501	10 i.v.	10 min.	2	2.6	2	0
Atropine	300 s.c.	2 min.	2	3.7	2	0
Banthine	5 i.v.	20 min.; 2 hrs.	2	3.2	2	0
Nembutal	75 i.v.+s.c.	1 min.†	2	3.8	2	0
Procaine	10 i.v.	1–30 min.	3	3.2	3	0
Neo-Antergan	15 i.v.	3 min.	2	2.6	2	0
SC-1950	10–20 i.v.	30 min.–2 hrs.	3	3.1	3	0

* 500 I.U. subcutaneously.
† 25 mg./kg., i.v. A total of 50 mg./kg., s.c., was given in divided doses at intervals after HCG to keep animal anesthetized for at least 6 hrs.

ganglionic blocker than tetraethyl-ammonium (TEA). It should be emphasized that in our hands neither TEA (Sawyer *et al.*, 1950), SC-1950, procaine nor Neo-Antergan (Sawyer *et al.*, 1951) has blocked neurogenic stimulation of LH-release.

RESULTS AND DISCUSSION

The response of the rabbit ovary to 500 I.U. of either HCG or PMS was extreme: every large follicle became ruptured or hemorrhagic and/or cystic. Such an array of hemorrhagic cystic follicles is seldom encountered following the mating stimulus. There were no exceptions to the rule of excessive luteinization with HCG, which was used only on mature rabbits; in fact, every animal ovulated. With PMS, one of 4 immature subjects failed to ovulate, but it revealed a well luteinized hemorrhagic follicle. One sample of PMS was apparently defective; one ml. of this preparation ("500 I.U./ml.") produced no observable changes in the ovaries of either experimental or control animals, all four of which were mature. Hence the results with this sample have not been included in the table.

The results with blocking agents are summarized in Tables 1 and 2. It is

quite apparent that the effects of neither HCG nor PMS are blocked by any of the anti-nervous drugs. There was no observable reduction in the number of ruptured follicles or in the degree of luteinization that was attributable to any of the 8 "blocking" agents employed.

Our results seriously question the hypothesis of Kehl, Molina and Douard that the nervous system is involved in the mechanism by which placental gonadotrophins induce ovulation in the rabbit. Their evidence would be more convincing if it had been shown, in the case of each "blocking" agent, that it was capable of preventing copulation-induced ovulation by blocking the nervous stimulus. Such a demonstration requires that the

TABLE 2. ATTEMPTS WITH ANTI-NERVOUS AGENTS TO BLOCK THE EFFECTS OF EQUINE GONADOTROPHIN (PMS) IN THE RABBIT

Drugs	Dosage (mg./kg.) and route	Interval between drug and PMS* injections	Number of rabbits	Average weight kg.	Results	
					Number ovulated	Number blocked by drug
—	—	—	6	3.7	6	—
Dibenamine	30 i.v.	1.5 hrs.	2	3.8	2	0
SKF-501	10 i.v.	12 min.	2	2.6	2	0
Atropine	300 s.c.	6–9 min.	2	3.6	2	0
Banthine	5 i.v.	3 min.	2	2.6	2	0
Nembutal	75 i.v. +s.c.	1 min.†	2	4.0	2	0
Procaine	20 i.v.	5 min.	3	3.8	3	0
Neo-Antergan	10, 15 i.v.	4 min.	2	2.7	2	0
SC-1950	15 i.v.	5 min.	2	2.2	2‡	0

* 500 I.U. subcutaneously.

† 25 mg./kg., i.v. A total of 50 mg./kg., s.c., was given in divided doses at intervals after PMS to keep animal anesthetized for at least 6 hrs.

‡ The immature ovaries of one of these 2 rabbits did not respond with ruptured follicles, but histological examination revealed marked luteinization of an hemorrhagic follicle.

agent (1) prevent ovulation if injected within a matter of seconds post coitum, and (2) allow ovulation if withheld until five minutes after copulation. Unless the latter requirement is met the drug might be preventing ovulation by a generalized debilitating action or by an effect on the mechanism of LH release subsequent to the arrival of the neurogenic stimulus. This second requirement has not been met in any case in the studies under consideration. We have tested three of the agents in question, procaine, Neo-Antergan and TEA (Sawyer *et al.*, 1950, 1951), without success in meeting the first requirement—injection within seconds post coitum did not interfere with ovulation.

If the drugs used by Kehl's group were not responsible for the failure of HCG and PMS, why did ovulation not occur in their experiments? Perhaps the most likely explanation, one which would account for the failure both of the coital and placental gonadotrophin stimuli, is a lack of maturity of the rabbits. No weights were given, and we have already suggested

(Sawyer *et al.*, 1951) that "blockade" of the coital stimulus by procaine might merely represent the well known incapacity of immature rabbits to ovulate in response to the mating stimulus. The suggestion finds confirmation in the statements (Molina and Douard, 1950) that "the ovary remains small" and "the blocking action [of TEA] is lasting since in one case the injection of extracts [PMS] to the same rabbit did not induce ovulation 14 days later." Since the ganglionic blocking action of intravenous TEA is transitory, lasting only about an hour (Moe and Freyburger, 1950), we consider this experiment a control showing that ovulation failed even in the *absence* of a blocking agent. It should be remembered that TEA does not block the coital stimulus under any circumstances (Sawyer *et al.*, 1950).

The present results are in accord with the finding of Hinsey and Markee that HCG is effective by some mechanism other than neurogenic stimulation of the hypophysis. Whereas our experiments do not deny a possible direct stimulation of hypophyseal secretion by placental gonadotrophins, considerable evidence points to a synergistic action of the latter with naturally secreted pituitary gonadotrophin as the probable mechanism under ordinary conditions. Supporting this concept are the experiments of Hill and Parkes (1931) and Hinsey and Markee (1933), who induced ovulation with HCG in the recently hypophysectomized mature rabbit, and the findings of Friedman (1932), Zondek (1935), and Tuchmann-Duplessis and Pfeiffer (1949), who have all reported stimulatory effects on direct application of HCG to the ovary. Williams (1945) finds no evidence in the immature rat of increased secretion of endogenous gonadotrophin on injecting PMS in dosages giving responses within the normal physiological range. It would appear that the burden of proof falls on those who consider that placental gonadotrophins exert their effects via pituitary stimulation.

SUMMARY

Human chorionic and equine gonadotrophins (HCG and PMS, respectively) induce ovulation in the rabbit, a species which ordinarily ovulates only after copulation-initiated neurogenic stimulation of the release of pituitary gonadotrophin. A nervous component in the mechanism by which the placental gonadotrophins stimulate ovulation is implied in a report that their actions are blocked by anti-nervous drugs such as procaine and Neo-Antergan. The present study tests this hypothesis by attempting to prevent the effects of HCG and PMS not only with procaine and Neo-Antergan but also with Dibenamine, SKF-501, atropine, Banthine and Nembutal. The latter are anti-adrenergic, anticholinergic and anesthetic agents whose effectiveness in blocking neurogenic stimulation of the adenohypophysis has been ascertained in the rabbit and/or the rat.

None of these agents, nor the ganglionic blocker SC-1950, had any effect on the actions of either HCG or PMS: all of the rabbits revealed ovarian stimulation. The results indicate that if the mechanism of placental gonadotrophin activity involves stimulation of the release of pituitary gonadotrophin, the excitatory effect is exerted directly on the hypophysis rather than indirectly via the nervous system.

Acknowledgments

We are grateful to the following corporations for generous gifts of the agents employed in this study: Ayerst, McKenna & Harrison Limited for A.P.L.; the Schering Corporation for Anteron; Smith, Kline and French Laboratories for Dibenamine and SKF-501; and G. D. Searle & Co., for Banthine and SC-1950.

REFERENCES

Cook, D. L., W. E. Hambourger and R. G. Bianchi: *J. Pharmacol. & Exper. Therap.* **99:** 435, 1950.

Dutta, N. K.: *Brit. J. Pharmacol.* **4:** 197. 1949.

Evans, H. M., and M. E. Simpson: Physiology of the gonadotrophins. In Pincus and Thimann: *The Hormones*, vol. 2, Academic Press, 1950.

Everett, J. W., C. H. Sawyer and J. E. Markee: *Endocrinology* **44:** 234. 1949.

Everett, J. W. and C. H. Sawyer: *Endocrinology* **47:** 198. 1950.

Friedman, M. H.: *Am. J. Physiol.*, **99:** 332. 1932.

Hambourger, W. E., D. L. Cook, M. M. Winbury and H. B. Freese: *J. Pharmacol. & Exper. Therap.* **99:** 245. 1950.

Hill, M. and A. S. Parkes: *J. Physiol.* **71:** 40, 1931.

Hinsey, J. C. and J. E. Markee: *Am. J. Physiol.* **106:** 48. 1933.

Kehl, R. and C. Molina: *Compt. Rend. Soc. de Biol.* **142:** 1522. 1948.

Kehl, R. and C. Molina: *Compt. Rend. Soc. de Biol.* **143:** 1175. 1949a.

Kehl, R. and C. Molina: *Ann. d'endocrinol.* **10:** 383. 1949b.

Leonard, S.: *Am. J. Physiol.* **108:** 331. 1934.

Moe, G. K. and W. A. Freyburger: *Pharmacol. Rev.* **2:** 61. 1950.

Molina, C. and T. Douard: *Compt. Rend. Soc. de Biol.* **144:** 530. 1950.

Nickerson, M.: *Pharmacol. Rev.* **1:** 27. 1949.

Reuse, J. J.: *Brit. J. Pharmacol.* **3:** 174. 1948.

Sawyer, C. H., J. W. Everett and J. E. Markee: *Endocrinology* **44:** 218. 1949.

Sawyer, C. H., J. E. Markee and J. W. Everett: *J. Exper. Zool.* **113:** 659. 1950.

Sawyer, C. H., J. E. Markee and J. W. Everett: *Am. J. Physiol.* In press, 1951.

Sawyer, C. H., J. E. Markee and W. H. Hollinshead: *Endocrinology* **41:** 395. 1947.

Sawyer, C. H., J. E. Markee and B. F. Townsend: *Endocrinology* **44:** 18. 1949.

Tuchmann-Duplessis, H. and C. A. Pfeiffer: *Compt. Rend. Soc. de Biol.* **143:** 356. 1949.

Westman, A. and D. Jacobsohn: *Acta Obstet. Gynec. Scand.* **22:** 16. 1942.

Williams, P. C.: *Endocrinology* **4:** 137. 1945.

Zondek, B.: (Cited in Zondek and Sulman, 1945) 1935.

Zondek, B. and F. Sulman: *Vitamins and Hormones.* **3:** 297. 1945.

DIETARY AND DOSAGE FACTORS AFFECTING THYROXINE INHIBITION OF ESTROGEN-INDUCED LIPEMIA IN THE CHICK

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IT HAS been repeatedly demonstrated that estrogen administration induces a marked lipemia in young chicks (Lorenz *et al.*, 1938) (Zondek and Marx, 1939). Fleischmann and Fried demonstrated that thyroxine could effectively inhibit the hypercholesterolemia seen in estrogen-treated chicks (Fleischmann and Fried, 1945). They also noted that this interference by thyroxine with the expected chemical changes in the serum did not alter the tissue-growth response to estrogen in the chick oviduct.

The present report includes observations which indicate: (a) that the degree of thyroxine inhibition of estrogen-induced lipemia in young chicks is quantitatively limited by the dosage of thyroxine, (b) that the thyroxine effect is not mediated by an excessive demand for factors of the B-complex created by the hyperthyroid state, (c) that the oviduct response to estrogen stimulation is quantitatively unaffected by simultaneous thyroxine administration, and (d) that the biotin activity of the serum previously reported by us to increase in the estrogen treated chick is suppressed to nearly normal levels by simultaneous thyroxine administration.

MATERIALS AND METHODS

New Hampshire Red female chicks were used throughout these studies. They were fed a commercial starting mash[1] and were housed in electrically heated brooders. Food was available at all times and mechanical controls provided 12 hours of illumination daily.

The excess quantity of B-complex factors was force-fed daily in aqueous suspension. Thyroxine (dl) was injected subcutaneously in 0.5 cc. alkalinized water. Estradiol benzoate was administered daily subcutaneously in 0.2 cc. corn oil.

The chicks were killed twenty-four hours after the last injection by bleeding from the heart. Total serum lipids were determined by a modification of Bloor's method in which a 2:1:1 mixture of alcohol, ether, and petroleum ether was substituted for the 3:1 alcohol-ether extractant.

The plasma was prepared from the heparinized blood by prompt centrifugation. One volume of plasma was mixed with 10 volumes of 3N H_2SO_4 and autoclaved for 1 hour at 15 lbs. pressure. The lipid fraction was separated from this hydrolysate by the method of Trager (Trager, 1947 (a)). Both the ether soluble and aqueous fractions were assayed

Received for publication March 22, 1951.

[1] Ralston's Purina Startena.

for biotin activity by the microbiological method employing Lactobacillus casei as the test organism (Landy and Dicken, 1942).

RESULTS AND DISCUSSION

Our observations (Table 1) fully confirm the findings of Fleischmann and Fried (1945) in that a decisive suppression of estrogen-induced lipemia is readily demonstrated. In addition, this interference by thyroxine does not

TABLE 1. EFFECT OF THYROXINE ON SUPPRESSION OF ESTROGEN-INDUCED LIPEMIA

Series	No. of chicks	Estradiol benzoate (mgms. daily)	Thyroxine (mcgms. daily)	Final body wt. (gms.)	Oviduct weight (mgms.)	Serum lipids (mg./cc.)	Water soluble biotin (mcgm./cc.)	Fat soluble biotin activity (mcgm./cc.)
A	6	1	0	253 ± 53	3435 ± 529	95 ± 14	.021 ± .004	.13 ± .03
	10	1	10	273 ± 88	4158 ± 27	86 ± 33	—	—
	7	1	25	288 ± 26	4438 ± 1287	84 ± 14	.012 ± .003	.12 ± .08
	10	1	50	243 ± 30	4146 ± 684	58 ± 8	.010 ± .003	.10 ± .01
	10	1	150	378 ± 40	2872 ± 212	66 ± 11	.010 ± .003	.10 ± .011
	9	1	200	346 ± 56	3342 ± 141	35 ± 8	—	—
	8	1	400	241 ± 7	4622 ± 654	23 ± 5	.006 ± .003	.07 ± .011
	10	0	0	274 ± 36	53 ± 14	20 ± 2	.002 ± 0.0	.012 ± .011
B	10	1	750	261 ± 28	2531 ± 272	22 ± 7	—	—
	12 (L)	1	750	242 ± 34	2426 ± 308	21 ± 2	—	—
	12 (B)	1	750	248 ± 34	2418 ± 311	20 ± 5	—	—
	10 (V)	1	750	257 ± 26	—	25 ± 9	—	—
	9	1	0	307 ± 21	—	103 ± 15	—	—
	10	0	0	285 ± 11	—	12 ± 1	—	—
C	10	1	0	236 ± 21	1159 ± 143	98 ± 17	—	—
	9 (V)	1	750	218 ± 39	1393 ± 214	19 ± 4	—	—
	9	1	750	235 ± 34	1458 ± 220	25 ± 8	—	—
	10	0	0	271 ± 30	61 ± 6	12 ± 6	—	—

Series "A" treated 8 days; "B" 6 days; "C" 5 days. All chicks were 27 to 30 days old when autopsied.
(L) Supplemented by daily feeding of .2 cc. commercial anti-pernicious anemia liver extract (15 units/cc.)
(B) Supplemented by daily feeding of 0.2 cc. B_{12} concentrate (10 mcgm./cc.)
(V) Supplemented by daily feeding of 1 cc. aqueous suspension containing: choline chloride, 320 mg.; Inositol, 160 mg.; calcium pantothenate, 8 mg.; niacin, 8 mg.; riboflavin 1.6 mg.; pyridoxine hydrochloride, 1.6 mg.; thiamine hydrochloride, 1.6 mg.; paramino-benzoic acid, 1.6 mg.; vitamin K, 0.8 mg.; biotin, .03 mg.; pteroylglutamic acid, .32 mg.

alter the tissue-growth response of the oviduct to the administered estrogen. This indicates a clear dissociation between the metabolic mechanisms involved in the peripheral tissue effect and the mobilization of serum lipids. We had previously demonstrated this dissociation in a converse manner since progesterone administration in the chick markedly reduces the oviduct response to administered estrogen but it does not interfere with estrogen-induced lipemia (Hertz, Larsen, Tullner 1947). It is therefore apparent that the effect of estrogens on the serum lipids depends upon a different metabolic pattern than that involved in the deposition of new tissue in the female genital tract following estrogen administration.

The close relationship between the degree of suppression of the lipemia and the dosage of thyroxine indicates that this effect is brought about through some direct quantitative action of the thyroxine upon the mechanisms involved in raising the serum lipids.

Drill and Overman (1942) demonstrated the remarkable protection from weight-loss afforded thyroid-intoxicated rats by administration of an excess of B-complex factors. In addition an increased requirement for folic

acid (Martin, 1947) and for vitamin B_{12} as well as for certain unidentified nutritional factors in liver has been demonstrated in the hyperthyroid rat (Ershoff, 1950).

Accordingly, we considered that the interference by thyroxine with estrogen-induced lipemia could be dependent upon the exhaustion in the hyperthyroid animal of some dietary trace factor essential for this metabolic response.[2] Our data indicate that an excess of the known B-complex factors or of a liver concentrate failed to affect the results. The supplementation of the diet with as much as 5% whole dried liver was also without effect. It would therefore appear that thyroxine mitigates estrogen-induced lipemia through some path other than thyrotoxic depletion of vital B-complex factors. The possibility still remains that some dietary factor other than those tested here may be involved.

Trager has demonstrated that the plasma of the actively laying hen contains a fat-soluble-factor (F.S.F.) which can replace biotin as an essential nutrilite for both the chick and Lactobacillus casei. (Trager, 1947b, 1948). We have previously reported that in estrogen-treated chicks there occurs a marked increase not only in F.S.F. but also in the true biotin content of the plasma. We noted also that although progesterone markedly suppressed the oviduct growth response to administered estrogen, it produced no significant depression in the high biotin and F.S.F. content of the plasma of the estrogen-treated bird (Hertz, Dhyse, Tullner, 1949). Our present data (Table 1, Series A) indicate that thyroxine inhibits this estrogen-induced elevation in plasma biotin and F.S.F. just as it reduces the lipemia. This suggests that these two distinct biochemical effects of estrogen may depend upon the same metabolic factors.

More adequate knowledge of the mechanisms involved in the effect of thyroxine upon serum lipids would be of practical as well as theoretical significance. There would seem to be at least some remote relationship between the quantitative sensitivity of the serum lipid level of the bird to thyroxine and the hypercholesterolemia encountered clinically in spontaneous or induced hypothyroidism (Salter, 1950). The demonstrated reduction in the chick of experimental atherosclerosis by thyroid feeding also suggests a general metabolic significance of these phenomena (Dauber *et al.*, 1949) (Horlick and Katz, 1948).

SUMMARY

Administration of thyroxine will suppress the marked hyperlipemia induced in young chicks by treatment with estradiol benzoate and the degree of suppression is proportional to the dosage of thyroxine. This suppression

[2] Grateful acknowledgement is made to the Upjohn Co. for supplies of B_{12} concentrate.

of the lipemia is not accompanied by any interference with the oviduct-growth effect of the administered estrogen. Oral administration of an excess of B-complex factors does not reverse the thyroxine inhibition of the lipemia, suggesting that the effect is not due to a thyrotoxic depletion of any of these dietary factors. The estrogen-induced increment in plasma biotin and fat-soluble factor (i.e., F.S.F. of Trager) is also suppressed by thyroxine.

REFERENCES

BLOOR, W. R.: *J. Biol. Chem.* **77:** 53. 1928.

DAUBER, D., L. HORLICK AND L. N. KATZ: *Am. Heart Journal* **38:** 25. 1949.

DRILL, V. A. AND R. OVERMAN: *Am. J. Physiol.* **135:** 474. 1942.

ERSHOFF, B. A.: *Proc. Soc. Exp. Biol. & Med.* **74:** 391. 1950.

FLEISCHMANN, W. AND I. A. FRIED: Endocrinology **36:** 406. 1945.

HERTZ, R., C. D. LARSEN AND WM. W. TULLNER: *J. National Cancer Institute* **8:** 123. 1947.

HERTZ, R., F. G. DHYSE AND WM. W. TULLNER: *Endocrinology* **45:** 451. 1949.

HORLICK, L. AND L. N. KATZ: *J. Lab. & Clin. Med.* **33:** 733. 1948.

LANDY, M. AND D. M. DICKEN: *J. Lab. & Clin. Med.* **27:** 1086. 1942.

LORENZ, F. W., C. E. ENTENMAN AND I. L. CHAIKOFF: *J. Biol. Chem.* **126:** 763. 1938.

MARTIN, G.: *Am. J. Dig. Dis.* **14:** 341. 1947.

SALTER, W. T.: The Chemistry and Physiology of the Thyroid Hormone in "The Hormones" Ed. by Pincus & Thimann; Academic Press, New York, 1950.

TRAGER, WM.: *Proc. Soc. Exp. Biol. & Med.* **64:** 129. 1947 (a).

TRAGER, WM.: *J. Exper. Med.* **85:** 663. 1947 (b).

TRAGER, WM.: *J. Biol. Chem.* **176:** 133. 1948.

ZONDEK, B. AND L. MARX: *Arch. internat. de pharm. et de therapie* **61:** 77. 1939.

THE EFFECT OF PITUITARY STALK-SECTION ON THE REPRODUCTIVE ORGANS OF FEMALE RATS[1]

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INTRODUCTION

IN CONTRAST to the well-known anatomical and physiological evidence of a functional relationship between the hypothalamus and the neurohypophysis, a controversy still exists as to whether the hypothalamus exerts direct control over the adenohypophysis. Much indirect evidence indicates that exteroceptive stimuli such as light, temperature, humidity, as well as olfactory, auditory, tactile and proprioceptive impulses may influence pituitary function. More direct evidence of such an influence has been obtained from experiments in which parts of the hypothalamus have been stimulated or damaged, the infundibular stalk has been transected or the hypophysis has been transplanted.

Section of the stalk provides a means for studying the results of interruption of possible neural paths from hypothalamus to pituitary, as well as of the hypophyseal portal blood vessels which, according to some, may carry a neurohumoral agent from diencephalic centers to the anterior pituitary. However, the importance of the alteration of the normal vascular supply of the pituitary *per se*, as a consequence of severing these vessels, does not appear to have been given adequate consideration.

What happens to the function of the anterior pituitary following stalk-section is at present poorly understood, since there has been a great diversity of findings and opinions among investigators who have used this approach.

The results of some of the previous studies are as follows: Harris (1937), Hinsey (1937) and Westman and Jacobsohn (1940) observed that section of the infundibular stalk in rabbits resulted in gonad atrophy and a loss of ability to mate. On the contrary, Hinsey and Markee (1933), and Brooks (1938, 1940) noted no alteration in structure or function of the ovaries or other genital organs. Brooks and Lambert (1939) found that al-

Received for publication March 31, 1951.

[1] This investigation was supported in part by a research grant from the National Institutes of Health, Public Health Service, as well as in part by a grant from the American Cancer Society on the recommendation of the Committee on Growth of the National Research Council.

though stalk-sectioned rabbits mated, they did not ovulate, and in a subsequent study Brooks, Bojar and Beadenkopf (1940) reported that 8 out of 21 rabbits showed uterine and ovarian atrophy. Gonad atrophy was also observed in dogs by Mahoney and Sheehan (1936) after application of a silver clip to the stalk and by Dott (1923) after stalk-section, but normal sex activity was reported by Keller and Hamilton (1937). Breckenridge and Keller (1948) observed later that 3 of 9 dogs showed pronounced uterine and gonad atrophy. Undisturbed estrous cycles have been reported by Dempsey (1939) in the guinea pig after stalk-section, whereas, irregular or absent vaginal cycles were sometimes observed by Leininger and Ranson (1943). Dandy (1940) reported a case of inadvertent section of the pituitary stalk in a young adult woman who had subsequent normal menstrual cycles, pregnancy and lactation. The results following stalk-section in the rat are in no better agreement as will be attested in a later section.

In the present study the effects of complete stalk-section in the rat have been reinvestigated using a surgical technique that permits excellent visualization of the stalk at the time of transection and assures a minimum of trauma to neighboring structures. Appropriate post-mortem examinations of the surgical area have also been carried out to determine the completeness of stalk-transection and the extent of regeneration of the hypophyseal portal vessels. Particular attention was paid to the blood supply, since it appears that the degree of vascular damage and possible repair may account in part for the variable results reported by previous investigators. Finally, the changes occurring in the function and structure of the reproductive organs have been carefully followed.

METHODS

Sixty-six rats taken from a stock of 94 adult female albinos of the Hisaw strain, in which vaginal smears had been recorded daily for nearly 3 weeks, were stalk-sectioned. The operative approach to the pituitary stalk was parapharyngeal and exposed first the ventral portion of the basisphenoid bone into the diploe of which bone wax was injected hypodermically. Using a dental drill, a long narrow mid-sagittal incision was made into the basisphenoid, taking care not to expose more than the rostral border of the pituitary gland. The use of warm bone wax prevented hemorrhage. A long tear was then made in the dura with a modified dental explorer immediately ventral to the stalk. The latter often bulged up through this tear. In any event, the borders of the stalk were clearly discernible as were the hypophyseal portal vessels which traverse this structure. It was then a simple matter to insert a fine hook beneath the stalk and by lifting up to sever it under direction vision. Hemorrhage was insignificant.

Control animals included 10 in which the stalk was exposed but not sectioned and 18 which were unoperated. The animals were fed fortified ground chow and kept in a room at 25° C. and illuminated 14 hours daily. The water intake of 30 stalk-sectioned and 12 control rats was measured daily during the experiment. Stalk-sectioned and sham-operated rats were killed with ether 19 to 54 days after operation. Unoperated control

animals were killed after data of vaginal smears and water intake had been recorded for 25 or more days.

At necropsy, the ovaries and uterus of each animal were inspected, removed and weighed. The ovaries and a sample of the vagina were fixed in 10% neutral formalin. Frozen sections were made from one ovary and stained with sudan IV while the other ovary and the specimen of vagina were imbedded in paraffin, sectioned at 7μ and stained with hematoxylin and eosin.

In one-third of the stalk-sectioned rats the calvarium was removed and the head fixed in Zenker's fluid for one week. The heads were decalcified in 5% trichloracetic acid and imbedded in paraffin. Serial sections were prepared in the sagittal plane at 7μ through the region of the pituitary, stalk and hypothalamus, and stained by the Mallory-Azan technique. The control, sham-operated, and remaining experimental animals were perfused with a 50% aqueous solution of Higgins' India ink in order to visualize the vascular supply of the pituitary. The ink was introduced by cannulation of the proximal aorta through the wall of the left ventricle. In half of the experimental animals perfused in this manner, the heads were fixed in 10% neutral formalin, imbedded in celloidin and cut alternately at 100μ and 10μ sections, through the regions noted above. The 10μ sections were stained by the Mallory-Azan method; whereas the 100μ sections were mounted unstained for study of the injected vessels. The heads of the remaining perfused animals were examined carefully with a low power dissecting microscope, the character of the pituitary blood vessels and the success of the stalk-section being checked. The pituitary gland was then removed, weighed, fixed in Zenker's fluid, imbedded in paraffin, sectioned serially at 7μ and stained by the Mallory-Azan method.

RESULTS

Fifty-two of the 66 stalk-sectioned rats survived the postoperative period and in 43 rats the completeness of stalk-section was verified by dissection or study of serial sections. In the remaining 9, the infundibular stalks were incompletely sectioned or the extent of the damage to the stalk could not be determined for technical reasons.

Vaginal smear: Severance of the pituitary stalk invariably produced a major disruption of normal estrous rhythm as revealed by examination of daily vaginal smears. Among the 43 rats so studied, 4 fairly distinct types of vaginal response were noted (fig. 1). 1) Complete absence of cornified smears was observed in 14 rats. 2) One or more days of cornified smears occurred in 18 rats within 5 days after operative date but not thereafter. In most of these, the cornified state was prolonged as much as 3 days, whereas, in others, the duration of the vaginal cornification was approximately normal. 3) Three rats showed one cornified smear 17 or more days after stalk-section. 4) Finally, in 8 instances, a cornified smear of one day's duration was observed on 2 to 5 separate occasions but these were irregularly spaced and did not appear before 20 days after operation. In all of these 4 categories leucocytes predominated in the smears in the days after stalk-section except for occasional days of mixed leucocytes and cornified cells or the rare instances of a predominantly cornified smear.

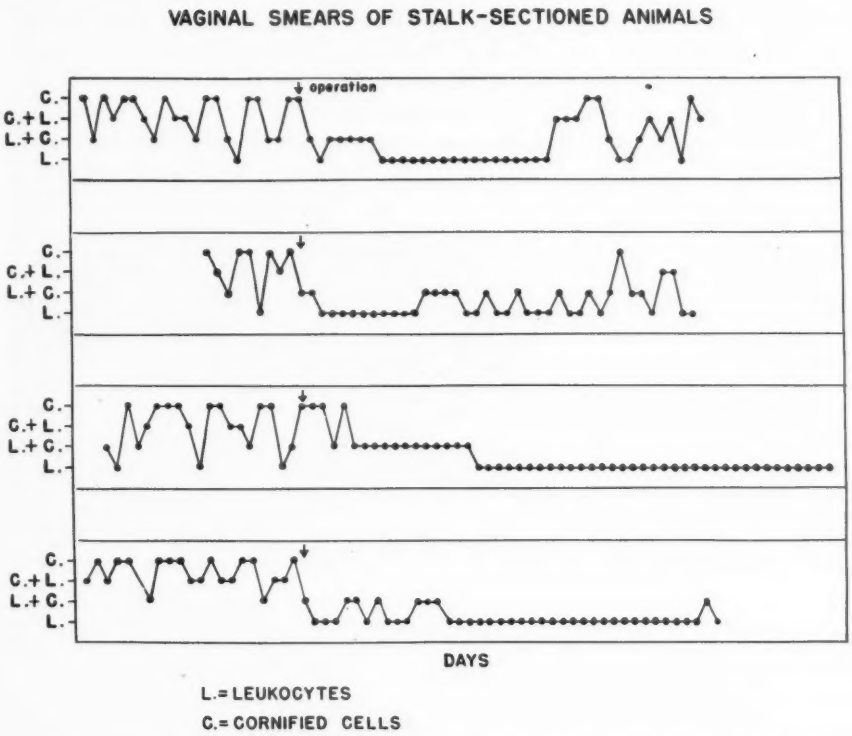


FIG. 1. Graphs of vaginal smears after stalk-section. From above down, the first subfigure exemplifies an instance showing more than one cornified smear late in the postoperative period; the second shows only one cornified smear after operation; the third, cornified smears immediately postoperatively and none thereafter; and the fourth, no cornified smears after operation.

The sections illustrated in figures 2–6 were stained with hematoxylin and eosin.

In those rats in which the stalk was incompletely sectioned, cornified smears were frequent, but the cycles were usually less regular than for the preoperative period. Sham-operated rats suffered no interruption of cycles although sometimes the cycles in the immediate postoperative period were prolonged.

Vagina: In 32 completely stalk-sectioned animals in which a leucocyte smear was recorded on the day of necropsy, histological study revealed vaginal epithelium displaying various degrees of development. In 8 of these the epithelium was atrophic consisting of only 2 layers of cells, the basal one of which was cuboidal or low columnar (fig. 2). A multicellular stratified epithelium, lacking cornification or mucification, typical of the

diestrous state was seen in 10 instances (fig. 4). Fourteen animals showed an intermediate condition in that the epithelium was 3 to 4 cells thick, non-squamous in character and diffusely infiltrated with leucocytes (fig. 3). In 8 rats having a smear of mixed leukocytes and epithelial cells at the time of death, the vaginal epithelium showed incipient cornification (fig. 5) in all but one instance. In the exception, desquamating cells and a stratum

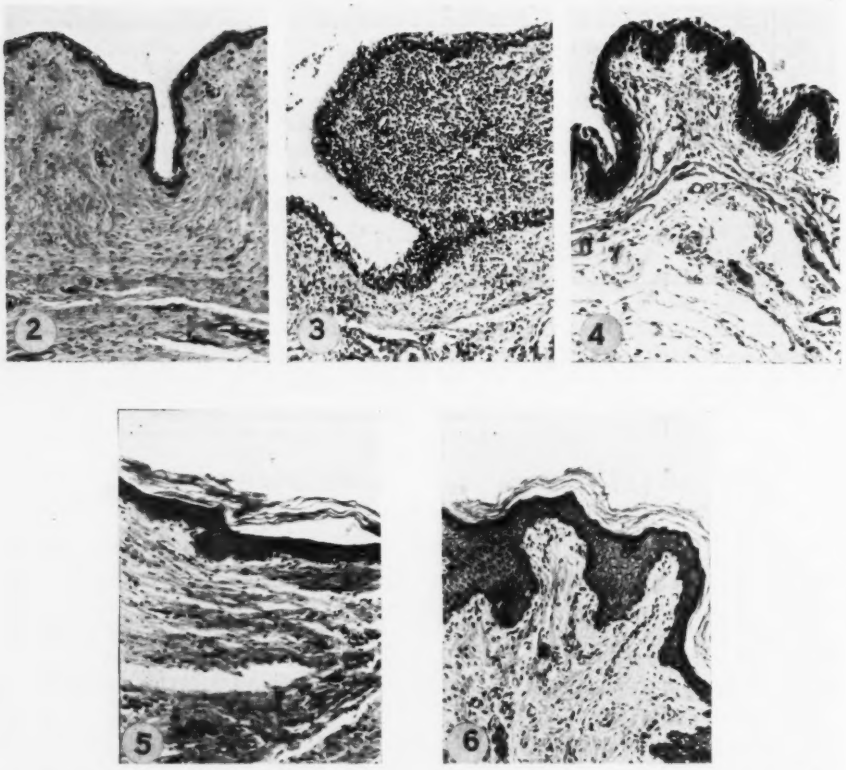


FIG. 2. Section of vagina of a stalk-sectioned rat showing extreme atrophy of epithelium. ×90.

FIG. 3. Vagina of a stalk-sectioned rat showing marked leukocytic infiltration of the lamina propria and moderate atrophy of the epithelium. ×90.

FIG. 4. Vagina of a stalk-sectioned rat showing a rather normal diestrous epithelium. ×90.

FIG. 5. Beginning cornification of vaginal epithelium of a stalk-sectioned rat in which the corresponding vaginal smear showed leukocytes and cornified cells. The epithelium, although thin, had been partially stimulated. ×90.

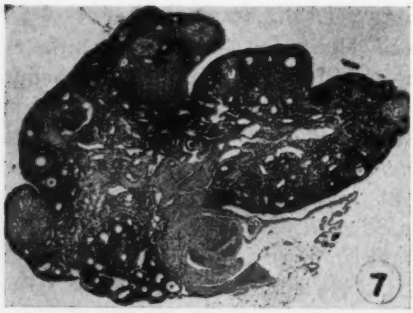
FIG. 6. Vagina of a stalk-sectioned rat which showed a cornified smear. The epithelium is thicker than in the previous animal but still thinner than normal. ×90.

granulosum and lucidum were noted; yet, the total thickness of the vaginal epithelium was less than normal. Finally, in 3 rats with completely cornified smears coinciding with the day of death, the vaginal epithelium resembled in histological features (fig. 6) that of normal animals in estrus, except that the epithelium was thinner. In contrast to these experimental results, a thick stratified epithelium, varying in histological detail according to the stage of the estrous cycle at time of death, was uniformly observed in the control and sham-operated rats.

Uterus: It was conspicuously apparent from gross inspection at necropsy that the uteri of the stalk-sectioned rats were smaller and less vascularized than those of the normal control and sham-operated animals. The average uterine weight was 123.6 mg. with a range of 74.0 to 185.0 mg. as compared to average weights of 321.8 mg. for unoperated controls and 334.7 for sham-operated ones. The range in the latter 2 groups was 222.0 to 453.0 mg. Stalk-sectioned rats which displayed at one time or another some vaginal cornification usually had uterine weights higher than those rats which showed no cornification but, invariably, well below the control values.

Ovary: The average weight of the paired ovaries of the 43 stalk-sectioned rats was 31.8 mg., the range being 12.9 to 49.6 mg. These weights contrasted with the average values of 61.4 mg. for normal rats and 60.2 mg. for sham-operated ones, the combined range being in these instances 49.4 to 77.0 mg. The ovaries in many of the stalk-sectioned rats had numerous peristing corpora lutea of the type characteristically seen in rats hypophysectomized post-puberally. These nonfunctional bodies obviously contributed significantly to the weight of these ovaries; hence, tended to obscure the meaning that weights of these organs generally have in terms of gonadotropic stimulation. This was especially evident from the fact that there was, with few exceptions, a pausity or complete absence of grossly visible large follicles in these ovaries irrespective of weight.

Noticeable atrophy was evident on examination of histological sections of the ovaries but the extent of these changes was variable. In extreme cases there was complete absence of vesicular follicles, corpora lutea and extensive loss of interstitial substance (fig. 7). In the majority of rats, large persistent corpora lutea and atrophic interstitial tissue were noted, both of which invariably showed little sudanophilia. Only few of these ovaries showed occasional small vesicular follicles (figs. 9 and 10). The primordial follicles, however, in these specimens, as in most of the others, were sudanophilic, suggesting that they were undergoing atresia. Another category consisted of specimens in which there were a few healthy appearing small vesicular follicles and some sudanophilic primordial follicles but no corpora lutea (fig. 8). Finally, the ovaries of animals which displayed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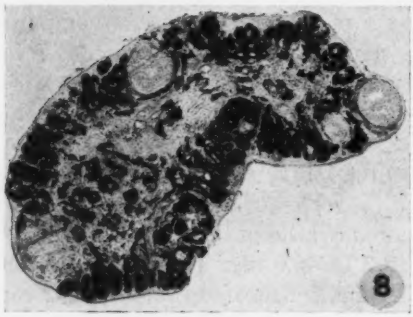
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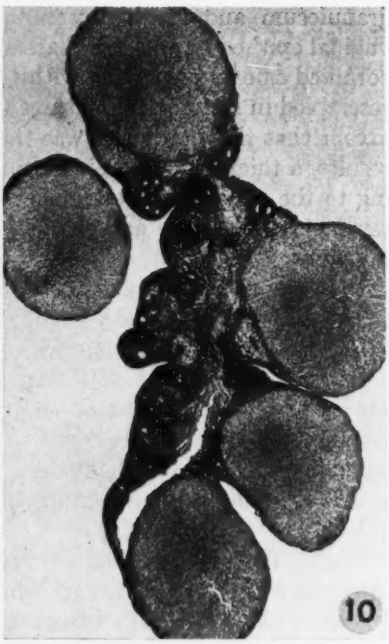
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vaginal signs of recent ovarian function showed mature graffian follicles. Often in these ovaries the persisting corpora lutea were found in various stages in degeneration (fig. 11).

Pituitary: Grossly, the pituitary glands of stalk-sectioned rats were smaller than normal, dull red in color rather than the usual pink, and friable. These glands showed profound morphological alteration as a result of stalk-section. Histological sections invariably revealed a striking post-operative accumulation of bundles of connective tissue in the central region of the anterior lobe. In the areas of the anterior pituitary immediately surrounding the central scar there were no acidophils or basophils. In general, this region contained small, uniform, rounded cells, chromophobic in character. Very few basophils and some acidophils were found in the most peripheral regions of the gland. Moreover, these chromophilic cells stained less intensely than did those in control pituitaries. Severe atrophy of the pars neuralis was noted in all instances. Lastly, it can be stated that there was no evidence in histological sections of any manipulative damage to the anterior pituitary in the course of operative transection of the stalk.

Some additional notes on the water intake and the pituitary circulation: The stalk-sectioned rats reported in this study uniformly showed a pronounced increase in water intake amounting in some cases to 5 to 6 times that of normal. No correlation could be drawn between degree of polydipsia and the condition of the vaginal smears.

Observations of the circulation of the pituitary which will be taken up in detail in a later communication emphasized a thorough and permanent interruption of the vessels which traverse the stalk. In only one instance was regeneration of these vessels suggested.

DISCUSSION

The experiments described above indicate that complete pituitary stalk-section in the adult female rat causes marked changes in the reproductive

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FIG. 7. Section of an ovary of a stalk-sectioned rat which showed no vaginal cycles. Note the extreme ovarian atrophy and lack of corpora lutea. Hematoxylin and eosin. ×30.

FIG. 8. Ovary of a stalk-sectioned rat stained with sudan IV. Note absence of corpora lutea and presence of numerous young follicles which are sudanophilic indicating atresia, and 4 small vesicular follicles. ×30.

FIG. 9. Ovary of a stalk-sectioned rat stained with sudan IV. Note large, persistent, sudanophobic corpora lutea, atrophic interstitial tissue, and few small vesicular follicles. ×30.

FIG. 10. Ovary of a stalk-sectioned rat. There are large, persistent corpora lutea, and follicular development is retarded. Hematoxylin and eosin. ×30.

FIG. 11. Ovary of a stalk-sectioned rat which had recent vaginal cycles. There are some persistent corpora lutea but only a few well-developed follicles. Hematoxylin and eosin. ×30.

cycle as measured by vaginal smears, gross and histological signs of atrophy of ovaries and of vaginal mucosa, and decrease in weight of the uterus. In general, the atrophy of the ovaries, although often severe, was seldom as extensive as in hypophysectomized rats. Conforming with these morphological results, there was evidence of hypoactivity rather than complete loss of ovarian function. The uterine weights and vaginal histology and smears reflected decreased rather than total loss of ovarian function.

These observed changes may be attributed to decreased production of gonadotropic hormones by the anterior pituitary. Such changes in hormonal production could be correlated with the histological changes noted in the pituitary glands consisting of central fibrosis and other alterations in cellular morphology, but never any direct damage to the anterior lobe.

Additional evidence of decreased anterior lobe function after stalk-sectioning has been obtained (Greep and Barrnett, 1950; Barrnett and Greep, 1950). This included severe atrophy of the testis and secondary sex organs of the male; hypoactivity of the thyroid gland and adrenal cortex under ordinary as well as altered environmental circumstances; and, finally, decreased pituitary gonadotropin activity in rats which were also castrated. Therefore, the ensemble of our results suggests that transection of the stalk of the pituitary causes panhypopituitarism in rats. As indicated in the results of these experiments, the hypopituitarism varied from severe to mild, with most of the animals showing a moderate hypopituitary state.

Our results conform to those of Westman and Jacobsohn (1937, 1938, 1943) and Brolin (1945) who reported female gonad atrophy in the rat after stalk-section. Richter (1933) also described prolonged diestrous and irregularities of vaginal cycles, although initially he had observed no alteration in sexual cycles of rats (Richter, 1930). On the other hand, our results are at variance with those of Uotila (1940) who reported that the ovaries of stalk-sectioned rats were normal and those of Dempsey and Uotila (1940) who concluded that the basic sexual rhythm of rats was unaltered by stalk-section. These observations have been criticized by Brolin (1945) and Westman, Jacobsohn and Hillarp (1943) who questioned the completeness of stalk-section. And according to Friedgood (1950) other conclusions may be drawn from the results of Dempsey and Uotila (1940), since only 9 of 27 rats showed normal cycles 2 to 3 months after stalk-section.

Hypoactivity of the anterior pituitary following section of the infundibular stem has been attributed to destruction of one or another of the components of the stalk (nerve pathways or vascular channels). As for interruption of possibly essential nerve pathways, there is little support in the literature for the view of hypothalamic innervation of the adenohy-

pophysis (see review by Harris, 1948). The possibility that the innervation of the adenohypophysis by sympathetic or parasympathetic fibers is of major significance for pituitary function is likewise open to question. Neuronal influences may play, nevertheless, an important part in the control of the adenohypophysis, albeit in an indirect manner by way of the vascular system, according to the neurovascular concept which has gained considerable favor in recent years (Brooks, 1938; Harris, 1948). This theory holds that nervous impulses from various areas in the hypothalamus cause the release of a neurohumor from the median eminence of the tuber cinereum. This substance is then transported to the anterior pituitary by the portal blood vessels of the stalk. Damage or stimulation of hypothalamic areas has lent some support to this hypothesis (Harris, 1948), but the necessary neuroanatomical and chemical evidence to establish firmly the existence of this pathway is, as yet, lacking.

According to the neurovascular concept, interruption of the stalk and, thereby, the portal vessels effectively blocks transport of the neurohumor to the pituitary and alters the function of the gland. The reappearance of vaginal cycles in stalk-sectioned rats Harris (1950) has recently observed, he attributed to regeneration of the portal blood vessels. Since the impulses for pituitary control of sexual rhythm arise, according to this author, from the hypothalamus, this diencephalic region has been termed the "sexual center."

There is another possible explanation of the effect of stalk-section on pituitary function which has emerged from the present studies. According to our results, complete stalk-section interrupts the major part of the vascular supply of the anterior lobe of the pituitary gland and causes ischemia therein which is reflected in diminished adenohypophyseal activity. In consequence of the ischemia one would expect cellular degeneration and scarring which, indeed, did occur as shown in our histological studies. Anatomical information on the arrangement and distribution of the blood supply of the stalk and anterior lobe supports this explanation. The anterior pituitary is supplied by portal vessels and arteries which descend in the stalk from the region of the tuber cinereum (Wislocki and King, 1936; Green and Harris, 1949). Direct observation of the flow of India ink in the blood vessels of the stalk (Green, 1947) substantiates these results. However, the vessels of the anterior pituitary gland do not become perfused with India ink when the stalk is transected just prior to injection; and, when the stalk is hemisected only the vessels of the contralateral half became filled (Barrnett and Greep, 1951). It is pertinent to note here that there was atrophy and scarring in the homolateral side of the anterior lobe of 3 rats in which the stalk was accidentally hemisected.

It is our contention, then, that the initial damage to the anterior lobe

of the pituitary gland attendant upon stalk-section is due to a vascular insult, and that the subsequent activity of the pituitary depends on the severity of the vascular damage and the later extent of revascularization. Unlike the recent results reported by Harris (1950), we have not, as yet, observed regeneration of the vessels of the stalk, a variance which may be due to differences in operative procedures. The pituitary stalk was approached through the temporal region by Harris and parapharyngeally by us. Necropsy observations of the operative site in our instances invariably showed that the stalk was buried in scar tissue and adherent to the anterior lip of the bony incision, a fact which could account for the lack of regeneration of vascular channels.

Finally, in connection with the discussion of the effect of alteration of the blood supply on pituitary function, the fact that endocrine functions other than those of the ovary are also rendered hypoactive (Barrnett and Greep, 1950) may be explained as resulting from ischemia rather than loss of a neurohumoral agent.

The results of our stalk-sectioned rats resemble in many ways some reports in the literature on rats in which the pituitary gland had been transplanted to another part of the body (Emory, 1936; Phelps *et al.*, 1939; Richter, 1937). In those instances, estrous cycles did not reappear even though the grafts appeared to be viable. Some pituitary functions may remain following transplantation as demonstrated in the recent work of MacDermott *et al.* (1950) who reported ACTH liberation by the graft in response to injection of epinephrine in animals showing gonad and adrenal atrophy. In still other reports of the effect of transplanting the pituitary gland, the maturation of ovarian follicles was observed, but the animals had no vaginal cycles (Haterius, 1937; Schweizer *et al.*, 1937). In contrast to the above reports Greep (1936) and May (1937) observed irregular and lengthened cycles following hypophysectomy and reimplantation of the pituitary into the sella turcica. Transplantation experiments are now in progress in our laboratory to study the differences in results when various sites are used for transplantation. In connection with the results of transplantation, the work of Gaillard (1942) and Martinovitch (1950) is very interesting, since they show that pituitary explants may be grown for more than 30 days in a synthetic medium and that later transplantation of these cultured pituitary glands supported growth. However, such transplants did not maintain organs dependent on pituitary tropic hormones, nor did they show basophils in histological sections.

This work does not deny the possible existence of a neurohumor which may well be a timing factor concerned with pituitary activity. It seeks only to point out that complete stalk-section in the rat may alter the

vascular supply of the pituitary gland so that its functional integrity is impaired.

SUMMARY AND CONCLUSIONS

Ovarian hypoactivity always appeared in rats after sectioning of the pituitary stalk by the parapharyngeal approach. Vaginal cycles were absent or irregular, ovarian and uterine weights were less than normal, and the vaginal epithelium was atrophic. Histological sections of the ovaries revealed general atrophy.

The hypopituitary state reflected by these changes correlated well with the fibrosis and cellular alterations observed in histological sections of pituitary glands of these animals. These results are believed to be due to ischemia as a result of interruption of those blood vessels to the pituitary which traverse the stalk. Ensuing pituitary function may be dependent on the extent of the vascular insult and of subsequent revascularization. Revascularization, whatever its source, was not due to regeneration of the portal blood vessels.

REFERENCES

BARRNETT, R. J. AND R. O. GREEP: *Science* **113:** 185. 1951.
BARRNETT, R. J. AND R. O. GREEP: *Anat. Rec. Supp.* **108:** 526. 1950.
BRECKENRIDGE, C. G. AND A. D. KELLER: *Am. J. Physiol.* **152:** 591. 1948.
BROLIN, S. W.: *Acta anat. Suppl.* **3:** p. 7. 1945.
BROOKS, C. MCC.: *Am. J. Physiol.* **121:** 157. 1938.
BROOKS, C. MCC.: *Proc. A. Research Nerv. & Ment. Dis.* **20:** 525. 1940.
BROOKS, C. MCC. AND E. F. LAMBERT: *Am. J. Physiol.* **128:** 57. 1939.
BROOKS, C. MCC., S. BOJAR AND W. G. BEADENKOPF: *Endocrinology* **27:** 873. 1940.
DANDY, W. E.: *J. Am. Med. A.* **114:** 312. 1940.
DEMPSEY, E. W.: *Am. J. Physiol.* **126:** 758. 1939.
DEMPSEY, E. W. AND U. U. UOTILA: *Endocrinology* **27:** 573. 1940.
DOTT, N. M.: *Quart. J. Exper. Physiol.* **13:** 241. 1923.
EMORY, F. E.: *Anat. Rec.* **66:** 253. 1936.
FRIEDGOOD, H. B.: Neuroendocrine and Psychodynamic Aspects of Endocrinopathies. Chapter 10 in Textbook of Endocrinology by R. H. Williams, W. B. Saunders Co., Philadelphia. 1950.
GREEN, J. D.: *Anat. Rec.* **99:** 21. 1947.
GREEN, J. D. AND G. W. HARRIS: *J. Physiol.* **108:** 359. 1949.
GREEP, R. O.: *Proc. Soc. Exper. Biol. & Med.* **34:** 754. 1936.
GREEP, R. O. AND R. J. BARRNETT: *Anat. Rec. Supp.* **108:** 525. 1950.
GAILLARD, P. J.: *Act. Sci. Industr., Paris, No. 923.* 1942.
HARRIS, G. W.: *Proc. Roy. Soc.*, London, s.B. **122:** 374. 1937.
HARRIS, G. W.: *Physiol. Rev.* **28:** 139. 1948.
HARRIS, G. W.: *J. Physiol.* **111:** 347. 1950.
HATERIUS, H. O.: *Cold Spring Harbor Symp., Quant. Biol.* **5:** 280. 1937.
HINSEY, J. C.: *Cold Spring Harbor Symp., Quant. Biol.* **5;** 269. 1947.
HINSEY, J. C. AND J. E. MARKEE: *Am. J. Physiol.* **106:** 48 1933.

KELLER, A. D. AND J. W. HAMILTON, JR.: *Am. J. Physiol.* **119:** 349. 1937.
LEININGER, C. R. AND S. W. RANSON: *Anat. Rec.* **87:** 77. 1943.
MCDERMOTT, W. V., E. G. FRY, J. R. BROBECK AND C. N. H. LONG: *Yale J. Biol. & Med.* **23:** 52. 1950.
MAHONEY, W. AND O. SHEEHAN: *Brain* **59:** 61. 1936.
MARTINOVITCH, P. N.: *Nature,* London **165:** 33. 1950.
MAY, R. M.: *Compt. rend. Soc. de biol.* 124: 920. 1937.
PHELPS, S., E. T. ELLISON AND J. C. BURCH: *Endocrinology* **25:** 227. 1939.
RICHTER, C. P.: *Brain* **58:** 76. 1940.
RICHTER, C. P.: *Am. J. Physiol.* **106:** 80. 1933.
RICHTER, C. P., AND J. F. ECKART: *Endocrinology* **21:** 481. 1937.
SCHWEIZER, M., A. A. CHARIPPER AND H. O. HATERIUS: *Endocrinology* **21:** 30. 1937.
UOTILA, U. U.: *Proc. A. Research Nerv. & Ment. Dis.* **20:** 580. 1940.
WESTMAN, A. AND D. JACOBSOHN: *Acta obst. et gynec. Scandinav.* **17:** 235. 1937.
WESTMAN, A. AND D. JACOBSOHN: Acta obst. et gynec. Scandinav. **18:** 109. 1938.
WESTMAN, A., D. JACOBSOHN AND N. HILLARP: Monatschr. f. Geburtsh. u. Gynäk **16:** 225. 1943.
WISLOCKI, G. B. AND L. S. KING: *Am. J. Anat.* **58:** 421. 1936.

STUDIES ON THE ROLE OF THE ADRENAL IN ANAPHYLAXIS AND HISTAMINE-SHOCK

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THE adrenal gland has been suspected of involvement in allergic conditions for over 20 years. In an early paper Wyman (1929) reported that adrenalectomized rats with adrenal transplants in the abdominal muscles were as susceptible to histamine poisoning and anaphylactic shock as adrenalectomized rats without transplants, but treatment with adrenalin somewhat alleviated this susceptibility. In a later paper, however, Wyman and Tum Suden (1937) reported the lethal doses of histamine to be as follows: normal rats, 100 mg. per kg. of body weight; adrenalectomized rats, 10 mg. per kg.; and adrenalectomized rats with adrenal transplants, 40 mg. per kg. Ingle (1937) also showed that autoplastic cortical transplants do not completely replace the function of the excised gland in rats. Perla and Gottesman (1931) found that adrenal cortical extract decreased the susceptibility of adrenalectomized rats to histamine and suggested that this property could serve as a basis for a cortical hormone assay.

Perry and Darcie (1946) reported that mice are not susceptible to histamine shock but do show anaphylactic shock under some conditions. By using massive doses of egg white as an antigen, shock symptoms were elicited in 92 per cent with death in 28 per cent. Since intact mice are so resistant to anaphylaxis, adrenalectomy in this species seemed to offer a further means of studying the role of the gland.

As it is difficult to maintain adrenalectomized mice in good health for a prolonged period of sensitization the following method was adopted. Male mice weighing 20–25 gm. were sensitized by 4 subcutaneous injections of 0.1 ml. of normal horse serum at 2 to 3 day intervals. Twelve days after the last injection they were adrenalectomized, after which they were given 0.9 per cent NaCl to drink. Two to 4 days later they were given an intraperitoneal injection of 0.1 to 0.2 ml. of horse serum. In a preliminary experiment, intraperitoneal administration of the shock dose was found to be as effective as the intravenous route. Antihistaminic compounds or adrenal extracts, when administered, were given intraperitoneally, $\frac{1}{4}$ to $\frac{1}{2}$ hour before the shock dose.

Controls for this work consisted of 50 sensitized mice which were treated but not adrenalectomized. One control animal died following the shock

Received for publication April 2, 1951.

dose, giving a survival rate of 98 percent. In contrast, only 153 out of 509 adrenalectomized mice survived, a rate of 30 per cent. As a further check on the anaphylactic nature of this reaction, all of a group of 28 non-sensitized adrenalectomized mice survived an intraperitoneal injection of horse serum.

Various adrenal extracts containing the steroidal fractions were administered to sensitized adrenalectomized mice to determine what factor was

TABLE 1. EFFECT OF ADRENAL EXTRACTS ON ANAPHYLAXIS IN ADRENALECTOMIZED MICE

Preparation	Cortisone equivalent	Dose per mouse	No. of mice	% survival
None	0		509	30
Aqueous Adrenal Extract, I	1 ml. = 1 mg.	.1 ml.	19	95
		.05	19	90
		.02	9	67
		.01	6	67
		.005	7	29
Lipoidal Adrenal Extract	1 ml. = 2 mg.	.2	15	60
		.1	15	53
		.05	15	53
Aqueous Adrenal Extract, II	1 ml. = ½ mg.	.1	20	45
		.025	10	30
		.01	20	25
Desoxycorticosterone Acetate	0	.5	7	14
		.25	7	14
		.2	15	33
		.1	9	11
		.05	14	36
		.025	8	25
		.01	15	20
		.002	13	8

responsible for the altered reaction. Adrenal extracts exerted a marked protective action (Table 1) but desoxycorticosterone acetate had no effect in the doses used. Since each ml. of extract I represents 100 gm. of beef adrenal gland an amount equivalent to about one gram of gland was necessary to secure significant protection in these animals. This extract had been shown to contain the equivalent of approximately 1 mg. of Kendall compound E per ml. as judged by its ability to cause glycogen deposition in adrenalectomized rats. The apparently greater efficacy of the aqueous extracts compared with the lipoidal extract was due probably to their injection in quickly absorbable form, as aqueous-alcohol suspensions, while the oily extract was not sufficiently available to the animal during the short period of the test.

The protective action of various antihistaminic compounds against anaphylactic shock was studied by a similar technique. Diphenhydramine in doses as low as 0.1 mg. per mouse afforded partial protection while 10 times this dose protected nearly all the animals (Table 2). Six other anti-

TABLE 2. EFFECT OF VARIOUS ANTIHISTAMINIC COMPOUNDS ON ANAPHYLAXIS IN ADRENALECTOMIZED MICE

Compound	Dose per mouse mg.	No. of mice	% survival
None		509	30
Diphenhydramine	1.0	10	90
	.5	10	70
	.25	10	60
	.2	30	60
	.1	40	48
	.05	10	10
	.01	10	30
Tripelennamine	.25	10	60
	.20	10	30
	.1	15	40
	.05	10	30
	.02	10	40
	.01	10	30
10-(beta-dimethyl amino ethyl) phenothiazine-5-oxide	.25	10	70
	.1	15	13
10-(beta-dimethyl amino ethyl) phenothiazine ethobromide	.25	10	70
	.1	15	27
10-(beta-dimethyl amino ethyl) phenothiazine methotheophyllinate	.25	10	60
	.1	15	40
10-(beta-dimethyl amino propyl) phenothiazine methochloride	.5	6	67
	.2	9	33
10-(beta-methyl hydroxy ethyl amino ethyl) phenothiazine	.25	10	40
	.1	15	40

histaminic compounds also showed activity. In general, the order of activity obtained in the adrenalectomized mouse was in good agreement with their ability to protect the normal guinea pig against anaphylactic shock.

The effect of injected histamine was also compared in intact and adrenalectomized mice. Preliminary tests showed that 0.5 mg. histamine diphosphate administered intraperitoneally to adrenalectomized mice was

TABLE 3. EFFECT OF VARIOUS ANTIHISTAMINIC COMPOUNDS ON HISTAMINE SHOCK IN ADRENALECTOMIZED MICE

Compound	Dose per mouse mg.	No. of mice	% survival
None		118	33
Diphenhydramine	.2	8	100
	.1	21	81
	.04	10	30
Tripelennamine	.04	10	80
	.02	10	50
10-(beta-dimethyl amino propyl) phenothiazine methochloride	.04	10	90
	.02	10	100

quite well tolerated but 2.0 mg. per mouse caused death in 70 per cent of the animals. The latter dose compared favorably with that reported by Wyman and Tum Suden (1937) and was used in all subsequent studies. Three of the antihistaminics used in the previous section of the study were tested and found to give nearly complete protection (Table 3). Comparison of the results of anaphylactic and histamine shock showed that the protective action of some of the antihistamines was much more marked against histamine shock than against anaphylactic shock although the survival rate of untreated adrenalectomized mice in both series was approximately the same.

DISCUSSION

These studies indicate that the mouse possesses an extremely active adrenal gland, as judged by the amount of beef adrenal extract necessary to restore the resistance of the adrenalectomized mouse to normal. This fact probably accounts for the resistance of the normal mouse to both anaphylactic and histamine shock.

Freed and Lindner (1941), considering the effectiveness of the adrenal steroids from another angle, found that corticosterone protected rabbits against increased capillary permeability following injections of leukotaxin but desoxycorticosterone did not. Our findings that whole adrenal extracts protect against anaphylaxis while desoxycorticosterone does not, further support the view that the neoglycogenic steroids participate in protection against conditions characterized by disturbances in permeability. The work of Hench (1949) and many others on the effects of Cortisone in human arthritis is concordant with this point of view.

SUMMARY

The susceptibility of mice to anaphylactic or histamine shock is increased markedly by adrenalectomy. Replacement therapy indicated that the neoglycogenic hormones play an important part in protection against anaphylaxis, while desoxycorticosterone was without effect.

The antihistaminics were also effective in protecting adrenalectomized mice against anaphylactic and histamine shock, but were considerably more active against the latter.

REFERENCES

Freed, S. C. and E. Lindner: *Am. Jour. Physiol.* **134**: 258. 1941.

Hench, P. S., E. C. Kendall, C. H. Slocumb and H. F. Polley: *Proc. Staff Meet. Mayo Clinic* **24**: 181. 1949.

Ingle, D. J.: *Am. Jour. Physiol.* **57**: 118. 1937.

Perla, David and J. M. Gottesman: *Proc. Soc. Exper. Biol. & Med.* **28**: 650. 1931.

Perry, S. M. and M. L. Darsie, Jr.: *Proc. Soc. Exper. Biol. & Med.* **63**: 453. 1946.

Wyman, L. C.: *Am. Jour. Physiol.* **87**: 29. 1929.

Wyman, L. C. and Caroline Tum Suden: *Endocrinology* **21**: 587. 1937.

ACTION OF HUMAN CHORIONIC GONADOTROPHIN UPON THE METABOLISM OF PHOSPHORUS IN THE THYMUS OF HYPOPHYSECTOMIZED IMMATURE RATS

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THE endocrine regulation of the metabolism of thymus is still incompletely understood. While the effect of steroid hormones and of pregnant mare serum (PMS) upon thymus is fairly well established, as far as we know, no data are available on the effect of human chorionic gonadotrophin (HCG) upon this organ. Experiments were therefore undertaken to ascertain the effect of HCG on the metabolism of phosphorus in the various fractions isolated from the thymus of hypophysectomized immature male rats.

MATERIAL AND METHODS

Hypophysectomized immature male rats of the Sprague-Dawley strain were used. The animals were purchased from Hormone Assay Lab. Inc., Chicago, Ill. U.S.A. Hypophysectomy was carried out at 21 days of age, body weight being between 35 and 45 g. During a 2 days' postoperative interval the animals were mailed by air. The rats were divided into 4 groups.

A: Control group, consisting of 8 animals, injected with saline.

B: 5 rats treated with a total of 0.1 I.U. of an electrophoretically purified homogenous HCG preparation.[1]

C: 5 rats treated with a total of 3.0 I.U. of HCG.

D: 5 hypophysectomized-gonadectomized animals, treated with a total of 3.0 I.U. of HCG.

Gonadectomy was performed on the third day following hypophysectomy. The injections were commenced on the fourth day after hypophysectomy and continued for 3 days, 1.0 ml., i.e. one-third of the total dose, being given subcutaneously at each injection. The animals were sacrificed 24 hours after the last HCG injection. 45 minutes prior to sacrifice, the animals were injected subcutaneously with 0.05 mC. of P^{32}/100 g. body weight. (Under our experimental conditions 1.0 mC. corresponds to 3.0×10^8 counts per minute.) The animals were anesthetized with ether and the thymus, adrenals, thyroid gland and ventral lobe of the prostate removed. The thymus was immediately dropped into a freezing mixture (dry ice—acetone), the frozen thymus was powdered and extracted in the cold room with 2 portions of ice-cold 10% solution of trichloroacetic acid (TCA). The TCA extracts were combined, completed to volume and aliquots were taken for the determination of phosphorus and radioactivity. In the TCA extracts only apparent free inorganic P and total acid soluble P were measured. The remaining tissues

Received for publication April 9, 1951.

[1] Gonadex-Leo, supplied through the courtesy of A. B. Leo, Hälsingborg, Sweden.

were extracted 3 times with a boiling mixture of ethanol and peroxide-free ether (3:1). The ethanol-ether extracts were combined, evaporated to dryness and hydrolyzed with conc. sulfuric acid. The P of this fraction is denoted as phospholipid-P. Finally the acid insoluble, ethanol-ether insoluble residual fraction, containing mainly nucleic acids, was digested with conc. H_2SO_4.

The adrenals and anterior prostate were weighed on a Mettler automatic balance, and hydrolyzed with conc. sulfuric acid. In these organs, as well in the thyroid, total P and radio-activity were measured.

Phosphorus was analyzed according to the method of Martin and Doty (1949). P^{32} was analyzed as inorganic P, precipitated in the form of magnesium ammonium phosphate as described by Lindberg (1946). All the P analyses and P^{32} measurements were carried out in duplicate.

RESULTS

The results are given in terms of specific activity. Specific activity (S.A.) is defined as the ratio of impulses per minute/μg. P. The specific activities of the fractions isolated from the thymus are presented in Table 1.

TABLE 1. SPECIFIC ACTIVITIES IN FRACTIONS ISOLATED FROM THE THYMUS OF HYPOPHYSECTOMIZED IMMATURE MALE RATS FOLLOWING THE ADMINISTRATION OF VARIOUS DOSES OF HUMAN CHORIONIC GONADOTROPHIN (HCG)

Inorganic P				Acid soluble Organic P (Total acid sol. P—Inorganic P)				Phospholipid P				Residual P		
A	B	C	D	A	B	C	D	A	B	C	D	B	C	D
55.6	37.6	17.9	32.3	16.3	12.3	4.6	14.5	3.64	0.26	0.36	0.30	0.58	0.38	0.47
48.9	40.0	15.4	25.4	10.0	14.9	17.3	6.3	5.05	0.80	0.34	0.19	0.62	0.60	0.33
27.7	42.1	16.0	21.4	5.1	13.3	7.2	5.5	1.29	0.51	0.54	0.44	0.83	0.42	0.30
51.9	38.6	17.3	24.4	5.2	14.3	9.6	5.4	4.35	0.29		0.55	0.54	0.40	0.32
54.5	41.4		22.3	26.7			14.5	5.11	0.72			0.79		0.32
35.5				11.9				3.78						
48.6				8.3				1.94						
34.6				14.0				2.04						
Mean:														
44.7	39.9	16.7	25.2	12.2	13.7	9.7	9.2	3.40	0.52	0.41	0.37	0.67	0.45	0.35

Specific activity = counts per minute/microgram P.
A = Controls
B = 3 × 0.033 I.U. of HCG
C = 3 × 1.0 I.U. of HCG
D = Gonadectomized, 3 × 1.0 I.U. of HCG

Inorganic phosphate shows a high S.A. both in the control group and in the group treated with the smallest dose of HCG, while the S.A. is greatly reduced in the groups injected with 3 I.U. of HCG. The difference between S.A. in groups A and B is statistically not significant, whereas the difference between A and C is significant at level of $p < 0.001$, and that between groups A and D, at level of $p < 0.01$.

The S.A. of the acid soluble organic P (i.e. the difference between total acid soluble P and inorganic P) is lower in groups C and D than in groups A and B, but in none of the cases is the difference significant.

The S.A. shows the most striking difference in the phospholipid fraction. Whereas the control group has an average S.A. of 3.40, this value is greatly reduced in the groups treated with HCG, being 0.52, 0.41, and

0.37 respectively. The difference between the control group and all of the HCG-treated groups is significant at level of $p < 0.01$. Thus treatment with HCG results in a greatly diminished turnover of the phospholipid fraction, and this effect can be demonstrated in gonadectomized animals as well as in non-gonadectomized ones. The values obtained in group B, i.e. the group treated with 0.1 I.U. of HCG, are of particular interest. This small dose has practically no effect upon the turnover of inorganic P or of acid soluble organic P, while it strongly reduces the turnover of phospholipid P. This fact seems to indicate that the primary effect of HCG is not directed upon the turnover of inorganic phosphate but upon that of the phospholipid fraction of this organ. It could be argued, however, against this assumption, that the increased metabolism—shown in the control group—is only a consequence of hypophysectomy and that HCG treatment causes no real inhibition but a mere normalisation of the phospholipid turnover. It was shown, however, by Fraenkel-Conrat and Li (1949) that the turnover of phospholipids is higher in the thymus of normal animals than in that of hypophysectomized ones.

Unfortunately, in the residual fraction of the control animals only radioactivity measurements could be carried out but no phosphorus analyses. However, in another control group of 4 animals, hypophysectomized on the same day, the residual fraction contained an average of 303 μg. P. If the S.A. values are calculated on this basis, a S.A. value of 0.74 is obtained which would be in agreement with the values obtained in group B. In the groups treated with 3.0 I.U. of HCG the specific activities were definitely diminished. The difference between groups B and C gives a value of $p < 0.05$, while the difference between groups B and D is significant at level of $p = 0.01$.

Since the residual fraction contains components with greatly differing functions, such as desoxyribonucleic acid (DNA), ribonucleic acid (RNA) and phosphoproteins, further studies are necessitated to find out which of these compounds is primarily inhibited by HCG. In this connection we should like to mention the findings of Hammarsten and Hevesy (1946). It has been shown by these authors that the turnover of RNA is always greater than that of DNA.

The average amounts of phosphorus and radioactivity found in the various fractions are presented in Figs. 1–2. It appears from Fig. 1 that the total amount of phosphate does not differ significantly in the various groups. The organs were not weighed but the relationship of total P would roughly indicate that the rate of involution in the hypophysectomized and hypophysectomized-HCG-treated groups is not differing significantly.

It was claimed by Greep and Jones (1950) that androgens act directly upon thymus, causing involution of this organ. Since, however, after

HCG treatment essentially the same results were obtained in hypophysectomized and in hypophysectomized-gonadectomized animals, the hypothesis that HCG would act through inducing androgen synthesis in the testicles can entirely be ruled out.

The question arises, however, whether HCG could not possibly exert its effect through a release of androgenic or related substances from the

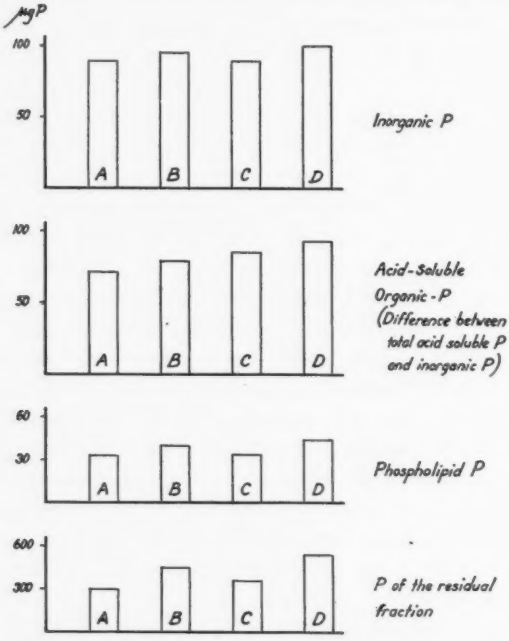

FIG. 1. Phosphorus in the various fractions isolated from thymus of hypophysectomized immature male rats.
Treatment: A: controls
B: 3×0.033 I.U. of HCG
C: 3×1.0 I.U. of HCG
D: 3×1.0 I.U. of HCG, gonadectomized.

adrenals. The amount of andromimetic substances produced in hypophysectomized animals under the influence of HCG can be determined by measuring the weight of the ventral prostate. In the control group the ventral prostate weighed 8.8 ± 0.39 mg. The hypophysectomized-gonadectomized group which was treated with a total of 3.0 I.U. of HCG exhibited a prostate weight of 7.8 ± 1.05 mg., while in the non-gonadectomized group, treatment with 3.0 I.U. resulted in a prostate weight of 18.9 ± 3.20 mg. These results agree with those of a previous paper (Diczfalusy, Holm-

gren & Westman, 1950), showing that even 60 I.U. of HCG could not induce any weight increase of the anterior prostate in hypophysectomized-gonadectomized rats. Thus it can be safely concluded that pure HCG in itself can not induce any release of andromimetic substances from the adrenals of hypophysectomized-gonadectomized animals.

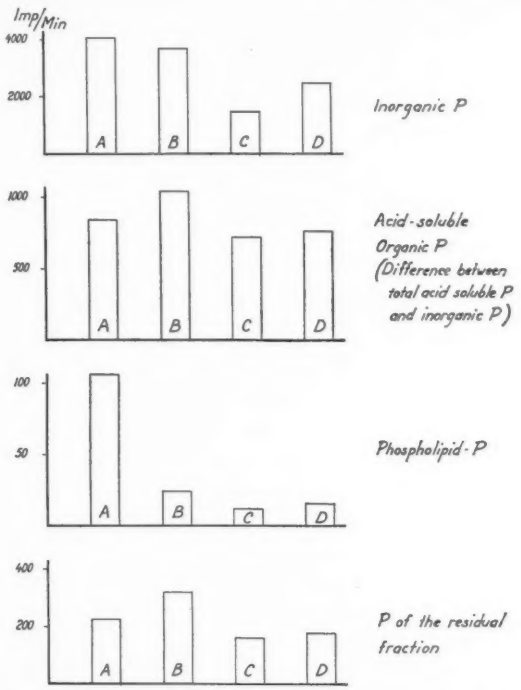

FIG. 2. Labelled phosphorus in the various fractions isolated from thymus of hypophysectomized immature male rats.
Treatment: A: controls
B: 3×0.033 I.U. of HCG
C: 3×1.0 I.U. of HCG
D: 3×1.0 I.U. of HCG, gonadectomized.

It was reported by several authors that ACTH causes involution of the thymus. Since, however, it was demonstrated by Li (1948) that a pure ACTH preparation had no effect on the secondary sex organs of hypophysectomized rats, it might be thought that under the influence of ACTH the adrenals produce steroid substances, which are not andromimetic in their action, but which can act upon the thymus, causing involution of this organ. That HCG would act in the same manner seemed

improbable, since even massive doses of HCG could not induce any increase in the adrenal weight of hypophysectomized-gonadectomized rats. However, in order to produce additional evidence against the assumption that HCG would act upon the metabolism of thymus through a stimulation of the adrenals, the adrenals of the animals treated with various doses of HCG were weighed and the P^{32}/mg. organ values determined. The results are given in Table 2. It appears from Table 2 that the P^{32}/mg. organ

TABLE 2. LABELLED PHOSPHORUS IN THE ADRENALS OF HYPOPHYSECTOMIZED IMMATURE MALE RATS FOLLOWING THE ADMINISTRATION OF HUMAN CHORIONIC GONADOTROPHIN (HCG)

Treatment		No. of rats	P^{32}/mg. wet weight
	Controls	8	16.8 ± 2.22
	3×0.0033 I.U. HCG	5	14.5 ± 1.18
	3×0.033 I.U. HCG	5	11.8 ± 2.55
	3×1.0 I.U. HCG	5	7.4 ± 1.03
Gonadectomized	3×1.0 I.U. HCG	5	6.7 ± 0.44

values decrease with increasing doses of HCG. Whether this phenomenon depends on a change in the size of the extracellular space, or on some other factors cannot be settled here. This problem will be discussed in another paper. Since, however, in some control animals, hypophysectomized at the same time and treated with moderate doses of ACTH, greatly increased turnover values were found, it can be stated that treatment with HCG does not result in an increased functional activity of the adrenals. That HCG could act upon the thymus via adrenal stimulation seems thus improbable.

It was recently reported by Fraenkel-Conrat and Li (1949) that the feeding of desiccated thyroid greatly reduces the turnover of phospholipids in the thymus of normal rats. It seemed thus necessary to ascertain whether the great reduction in the turnover of phospholipids after HCG administration does not depend on a possible activation of the thyroid gland. Therefore the S.A. of the thyroid gland was also determined. Results are given in Table 3.

TABLE 3. SPECIFIC ACTIVITIES IN THE THYROID OF HYPOPHYSECTOMIZED IMMATURE MALE RATS FOLLOWING THE ADMINISTRATION OF HUMAN CHORIONIC GONADOTROPHIN (HCG)

Treatment		No. of rats	P^{32}/μg. P
	Controls	8	8.6 ± 1.07
	3×0.033 I.U. HCG	5	4.5 ± 0.94
	3×1.0 I.U. HCG	5	3.9 ± 1.17
Gonadectomized	3×1.0 I.U. HCG	5	4.7 ± 0.57

The administration of HCG results in a diminished S.A. in the thyroid. similarly to the findings in the adrenals. This result speaks against the assumption that HCG might act through an activation of thyroid function. In this connection we should like to mention that according to Borell and

Holmgren (1949) even the slightest increase in the functional activity of the thyroid gland is parallelled by an elevated S.A. value.

DISCUSSION

The effect of hypophysectomy upon thymus is still a matter of dispute. Houssay and Lascano-Gonzales (1934) report frequent but not constant involution following the ablation of the pituitary, which is in accordance with previous investigations. Similarly, administration of pituitary extracts or implantation of the whole pituitary led to greatly diverging results. Uyldert and Freud (1938) report an increase in thymus weight in hypophysectomized animals following treatment with a pituitary extract practically free of gonadotrophic and thyreotropic activity. It must be pointed out however that the preparation of Uyldert and Freud contained growth hormone. This fact becomes especially important in the light of the recent findings of Fraenkel-Conrat and Li, who showed that pure growth hormone caused a definitely increased turnover of nucleic acids and phospholipids in the thymus of hypophysectomized rats.

On the other hand, it was reported by several investigators that the administration of ACTH causes involution of the thymus (Evans, Moon, Simpson and Lyons, 1938, Yoffey and Baxter, 1946, Simmonet, 1947). Furthermore it has been shown by Evans and Simpson (1934) that the administration of pregnant mare serum gonadotrophin (PMS) causes a definite involution of the thymus in hypophysectomized animals while in castrated-hypophysectomized animals no such effect could be obtained. It is also well established that both androgens and estrogens cause rapid involution of the thymus (Greep and Jones, 1950). It was therefore generally assumed that growth hormone—which might be identical with the so called thymotropic hormone—stimulates the thymus, whereas "purified gonadotrophic and adrenotrophic preparations cause thymic involution in proportion to the steroid hormone production which they elicit in the gonads and adrenals respectively" (Selye, 1947).

However, in view of the findings presented in this paper it seems reasonable to assume that HCG acts directly upon the metabolism of thymus—at least in hypophysectomized animals—by inhibiting the turnover of phospholipids in this organ.

It should therefore be taken into consideration, that—besides their target organs—gonadotrophic hormones might directly influence the metabolism of other organs.

SUMMARY

1. Small doses of electrophoretically purified homogeneous human chorionic gonadotrophin (HCG) decrease the turnover of phospholipids in the thymus of hypophysectomized immature male rats.

2. The inhibitory effect of HCG is not mediated through the gonads; in the absence of the testicles the same results are obtained.

3. Evidence is presented that HCG does not act by stimulation of the adrenals.

REFERENCES

BORELL, U. AND H. HOLMGREN: *Acta endocrinol.* **3:** 331. 1949.

DICZFALUSY, E., H. HOLMGREN AND A. WESTMAN: *Acta endocrinol.* **5:** 43. 1950.

EVANS, H. M., H. D. MOON, M. E. SIMPSON AND W. R. LYONS: *Proc. Soc. Exptl. Biol. Med.* **38:** 419. 1938.

EVANS, H. M. AND M. E. SIMPSON: *Anat. Rec.* **60:** 423. 1934.

FRAENKEL-CONRAT, J. AND C. H. LI: *Endocrinology* **44:** 487. 1949.

GREEP, R. O. AND I. C. JONES: *Rec. Progress in Hormone Res.* **5:** 197. 1950.

HAMMARSTEN, E. AND G. HEVESY: *Acta Physiol. Scand.* **11:** 335. 1946.

HOUSSAY, B. A. AND J. M. LASCANO-GONZALES: *Compt. Rend. Soc. Biol.* **117:** 463. 1934.

LI, C. H.: In *Rec. Progress in Hormone Res.* **3:** 246. 1948.

LINDBERG, O.: *Arkiv f. Kemi* **23A**, No. 2. 1946.

MARTIN, J. B. AND D. M. DOTY: *Anal. Chem.* **21:** 965. 1949.

SELYE, H.: Textbook of Endocrinology, Montreal 1947, p. 684.

SIMMONET, H.: *Bull. Acad. Med.* **131:** 245. 1947.

UYLDERT, J. E. AND J. FREUD: *Acta brev. Neerl. Physiol.* **8:** 188. 1938.

YOFFEY, J. M. AND J. S. BAXTER: *J. of Anat.* **80:** 132. 1946.

PREVENTION BY SOMATOTROPHIN OF THE CATABOLISM WHICH NORMALLY OCCURS DURING STRESS

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THE first papers on the general-adaptation-syndrome (G-A-S) had already stressed the fact that during exposure to stress, while the adrenal cortex enlarges, the gonads tend to undergo atrophy and somatic growth is diminished or abolished. Concurrently, in lactating animals, milk secretion decreases. These changes have been tentatively ascribed to a "shift in anterior-lobe hormone production," with a great predominance of ACTH production at the expense of other anterior-pituitary hormones. It was assumed that during times of great stress the hypophysis would preferentially produce the life-maintaining ACTH, rather than other less urgently required hormones, such as: follicle stimulating hormone (FSH), luteinizing hormone (LH), luteotrophic hormone (LTH) and somatotrophic hormone (STH). This view appeared to receive confirmation from experiments showing that the gonadal atrophy induced by various stressor agents could be effectively prevented by comparatively small doses of purified gonadotrophic hormones (Selye, 1936, 1939a, 1939b, 1950). Evidently, the involution of the gonads during stress was not due to an insensitivity of their cells to gonadotrophic substances, but rather to a deficient production of the latter. At that time sufficiently purified preparations of LTH and STH were not yet available to perform comparable experiments concerning the mechanism of the lactation and somatic growth inhibiting effects of stress.

In order to clarify this point, at least concerning the inhibition of growth, Ershoff (1951) has now exposed immature rats to the stressor action of cold; some of the animals received no hormone treatment, while others were given daily injections of somatotrophin. He argued that if the "shift in anterior-lobe hormone production" does in fact result in a diminished STH production, "then providing increased amounts of somatotrophin might be expected to counteract, at least in part, the growth retardation of immature rats maintained at low environmental temperatures." Since under these conditions STH failed to correct the growth inhibition, he concluded "that impaired formation or release of somatotrophin is not the cause of the growth retardation of immature rats under conditions of low environmental temperature."

Received for publication April 18, 1951.

Chronic exposure to cold differs from most other stressor agents in that it is particularly prone to produce renal and connective tissue changes similar to those elicited by heavy overdosage with STH (Selye, 1950, 1951). Furthermore, in the above mentioned experiments, very young rats (average initial body weight 57 gm.) were used and at this early age the growth impetus of the rat is so great that body weight increase continues even after complete hypophysectomy (Selye, 1949). We therefore decided to repeat these experiments using more mature rats and another stressor agent.

EXPERIMENTAL PROCEDURE AND RESULTS

32 female piebald rats, having an initial average body weight of 148 gm. were subdivided into four groups of eight animals each. Group I acted as untreated controls. Group II received 6 mg. of a highly purified STH preparation daily in the form of three subcutaneous injections of 2 mg. each, dissolved in 0.1 cc. of saline and given at eight hour intervals ("Somatrofin" of Frank W. Horner Ltd., Montreal, Lot no. 910-F 5116). Group III was given 0.5 cc. of turpentine, subcutaneously, every day during the first five days and then every second day during the rest of the experiment. Group IV was simultaneously treated both with STH as group II and with turpentine as group III, care being taken to inject the two substances in different parts of the body.

The experiment was terminated after eight days, the results being summarized in Table 1.

TABLE 1. SUMMARY OF BODY WEIGHTS OF RATS TREATED WITH STH AND TURPENTINE, SINGLY OR IN COMBINATION

Groups		I Controls	II STH	III Turpentine	IV STH + Turpentine
Body weight	Initial	148 ± 2.0	148 ± 2.4	148 ± 1.6	148 ± 1.6
	Final	152 ± 1.4	173 ± 6.4	137 ± 4.6	158 ± 3.3

It will be noted that turpentine (group III) not only inhibited the growth rate, but actually caused a loss of body weight in otherwise untreated animals, STH considerably promoted growth (group II) and at the same time completely prevented the catabolic action of turpentine (group IV).

A detailed morphologic study concerning the effects of STH upon the individual organ changes characteristic of the G-A-S, will be published elsewhere. Let us merely point out here, that the intense thymic and splenic involution caused by the multiple turpentine injections was completely prevented by simultaneous treatment with STH, in the present experimental series.

DISCUSSION

Under our experimental conditions STH successfully counteracted the growth-inhibiting and catabolic actions of the stress, which are occasioned by multiple, abscess-producing, subcutaneous turpentine injections. This does not prove that the inhibition of growth and the tissue catabolism, seen during the G-A-S, are due to a diminution of STH production. ACTH,

cortisone and various non-specific stressors are notoriously capable of causing catabolism even after hypophysectomy, when they could obviously not act by diminishing STH secretion.

Some observations suggest that, under certain conditions at least, stress may even be accompanied by an abnormal increase in STH production (Selye, 1951). Indeed, in view of the recently demonstrated infection-inhibiting effect of STH, (Selye, 1951a) such a rise would appear to be teleologically useful. However, the present experimental series demonstrates that under conditions of intense stress with weight loss, when ACTH production is greatly augmented, STH secretion becomes insufficient to prevent catabolism. This catabolism of the G-A-S, could in part also result from a loss of responsiveness to the anabolic actions of STH; yet this irresponsiveness is certainly not complete, since exogenous STH supplements can manifestly overcome it.

SUMMARY

The inhibition of growth and the loss of body weight, normally induced in rats by multiple sterile turpentine abscesses, can be completely prevented by adequate doses of somatotrophic hormone (STH).

Acknowledgements

This investigation was subsidized by a grant from the National Research Council of Canada. The author is indebted to Dr. L. Mitchell of Frank W. Horner Ltd., Montreal, for the "Somatrofin" used in these experiments.

REFERENCES

ERSHOFF, BENJAMIN H.: *Endocrinology* **48:** 111. 1951.
SELYE, HANS: *Canad. M.A.J.* **34:** 706. 1936.
SELYE, HANS: *Endocrinology* **25:** 615. 1939.
SELYE, HANS: *Proc. Amer. Physiol. Soc.* Toronto, April 1939.
SELYE, HANS: Textbook of Endocrinology, *Acta Endocrinologica Inc.*, Montreal 1949.
SELYE, HANS: "Stress," *Acta Inc., Medical Publ.*, Montreal 1950.
SELYE, HANS: *Brit. M. J.* Feb. 10, p. 263. 1951.
SELYE, HANS: Symposium on steroids, in Exp. and Clin. Practice, Blakiston & Co., New York. 1951a.

INHIBITION OF THE GAMETOKINETIC RESPONSE IN MALE "RANA PIPIENS" WITH ADRENOLYTIC DRUGS

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ROBBINS and Parker (1949) have demonstrated that the male amphibia, Xenopus laevis and Rana pipiens, respond to the subcutaneous administration of epinephrine (0.01 to 0.001 mg.) by the emission of spermatozoa. The Xenopus laevis proved reactive throughout the year while the Rana pipiens was considerably less sensitive to epinephrine during the summer months of June, July and August. Hypophysectomy failed to alter the sensitivity of the male Xenopus laevis and Rana pipiens to epinephrine.

Preliminary studies by Greenblatt, Clark and West (1949) indicated that the gametokinetic response to epinephrine and chorionic gonadotropin in Rana pipiens could be partially or completely blocked by the adrenolytic drug, piperidylmethyl benzodioxane. Having previously noted (Greenblatt, Clark and West, 1950) that the gametokinetic response in the male frog, Rana pipiens, seemed to depend primarily on the FSH factor in gonadotropins, the authors thought that further study of the physiologic mechanism producing the adrenergic release of spermatozoa was warranted.

In rather extensive studies we have found that several representative adrenolytic drugs will block the release of spermatozoa by the male Rana pipiens if injected prior to the injection of chorionic gonadotropin, pituitary gonadotropin, human pregnancy urine, levo-epinephrine, levo-arterenol, and epinephrine.

MATERIALS AND METHODS

Adult male frogs (Rana pipiens) weighing from 25 to 45 grams were used in this study. It was impossible to conduct experiments during the summer months as the sensitivity of the male Rana pipiens to chorionic gonadotropins is markedly reduced during June, July and August (Soucy, 1950; Waldrop and James, 1950; Greenblatt, Clark and West, 1950).

All of the test solutions were injected into the dorsal lymph sac of the male frog; following injection of the test substances the cloacal aspirate was examined microscopically for the presence of spermatozoa according to the technic described by Wiltberger and Miller (1948), Miller and Wiltberger (1948) and Robbins and Parker (1948).

The adrenolytic drugs employed in these experiments were: Benodaine Hydrochloride (Merck), Compound S.K.F. 688-A (N-Phenoxyisopropyl-B-Benzyl-Beta-chlorethyl-

Received for publication April 19, 1951.

amine Hydrochloride—Smith, Kline and French), Dibenamine Hydrochloride (Smith, Kline and French), Priscoline Hydrochloride (Ciba) and Compound C.C.K. 179 (Ergotoxine containing a mixture of dihydroergocristine, dihydroergokryptine, and dihydroergocornine—Sandoz). Preliminary experiments were conducted to determine the dosage which would block the gametokinetic response and yet cause no toxic effects on the frog. In testing the adrenergic blocking effect all of the adrenolytic drugs were injected about 30 seconds prior to the injection of the control gonadotropic or sympathomimetic substance.

Several gonadotropic substances and sympathomimetic drugs were studied: chorionic gonadotropin (Pregnyl-Roche Organon, and human pregnancy urine), pituitary gonadotropin (Gonadophysin-Searle), levo-epinephrine bitartrate (Sterling-Winthrop), levoarterenol bitartrate (Sterling-Winthrop), and epinephrine hydrochloride (Adrenalin-Parke Davis).

INTERPRETATIONS AND RESULTS

As shown in Tables 1 and 2, the gonadotropic-induced release of spermatozoa was inhibited in a large number of frogs by the prior injection of an adrenolytic drug. Failure of Benodaine Hydrochloride to inhibit gameto-

TABLE 1. INHIBITION OF GONADOTROPIC RESPONSE WITH ADRENOLYTIC DRUGS

Adrenolytic Drug	50 I.U. chorionic gonadotropin		1 R.U. pituitary gonadotropin	
	Positive frogs	Negative frogs	Positive frogs	Negative frogs
———	30	0	22	6
2.5 mg. Benodaine Hydrochloride	3	20	0	10 (October, February)
3.0 mg. Benodaine Hydrochloride	—	—	7	3 (May)
2.0 mg. SKF 688-A*	6	6	—	—
3.0 mg. SKF 688-A*	12	15	—	—
2.0 mg. CCK-179†	5	9	—	—
4.0 mg. CCK-179†	—	—	3	14

* N-Phenoxyisopropyl-B-Benzyl-N-chlorethylamine Hydrochloride.
† Mixture of equal parts of Dihydroergocornine, Dihydroergocristine and Dihydroergokryptine.

TABLE 2. INHIBITION OF GONADOTROPIC RESPONSE WITH ADRENOLYTIC DRUGS

Human pregnancy urine			0.5 mg. Benodaine HCl		2.0 mg. Benodaine HCl		2.5 mg. Benodaine HCl		2.0 mg. Compound CCK-179*		4.0 mg. Compound CCK-179		2.0 mg. Dibenamine HCl		10 mg. Priscoline HCl	
	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
1 cc.	3 2	0 0			3 0	1 2										
2 cc.	1	0							0	1	0	1				
3 cc.	1 2	0 0	0	1			0	1								
4 cc.	1	0					2	0								
5 cc.	1 2 3 2	0 0 0 0	0 1	1 0	0	2	0 2 1	2 0 0					1	1	0	1

* Ergotoxine—mixture of equal parts of dihydroergocornine, dihydroergocristine and dihydroergokryptine.

kinesis on injection of pregnancy urine in 4 cases can probably be attributed to a higher chorionic gonadotropin titer in those urines. Benodaine Hydrochloride in 2.5 mg. doses was more effective in blocking the response to commercial chorionic gonadotropin than 3.0 mg. of the Beta-chlorethylamine S.K.F. 688-A. Control frogs showed a uniformly positive response to 50 I.U. of chorionic gonadotropin and over 75% gave a positive response to 1 R.U. of pituitary gonadotropin. Benodaine hydrochloride exhibited decreased gonadotropic blocking effect in May as compared with the fall and winter months; this suggests seasonal variation in the emission of spermatozoa. C.C.K.-179 in dosages of 2.0 mg. and 4.0 mg. exerted better gonadotropic blockade than did 2.0 mg. and 3.0 mg. of S.K.F. 688-A.

The adrenergic-induced release of spermatozoa by epinephrine hydrochloride, levo-epinephrine and levo-arterenol was inhibited in a large per-

TABLE 3. INHIBITION OF ADRENERGIC RESPONSE WITH ADRENOLYTIC DRUGS

Adrenolytic drug	0.005 mg. l-epinephrine bitartrate		0.01 mg. l-epinephrine bitartrate		0.05 mg. l-arterenol bitartrate		0.1 mg. l-arterenol bitartrate		0.01 mg. adrenalin		0.02 mg. adrenalin		0.05 mg. adrenalin		0.005 mg. adrenalin	
	No. frogs															
	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg
—	34	15	8	0	8	2	16	4	18	1	9	1	9	1	5	0
2.5 mg. Benodaine hydrochloride	7	39			1	7			7	7						
1.0 mg. CCK-179			3	1												
2.0 mg. CCK-179			0	4												
4.0 mg. CCK-179			2	2					2	2					3	
10 mg. Dibenamine hydrochloride											5	12				
12.5 mg. dibenamine hydrochloride							4	14								
10.0 mg. priscoline hydrochloride													2	8		

centage of frogs by the prior injection of 2.5 mg. of Benodaine Hydrochloride as shown in Table 3. Compound C.C.K. 179 (mixture of the alkaloids of ergotoxine) in doses of 1.0–4.0 mg., Dibenamine Hydrochloride (10.0 mg., 12.5 mg.) and Priscoline Hydrochloride (10 mg.) caused adrenergic blockade in a fair percentage of cases. The approximate minimal dose of levo-epinephrine (0.005 mg.) and of epinephrine hydrochloride (0.005 mg.) necessary to provoke gametokinesis was ten times less than the approximate minimal dose of levo-arterenol (0.05 mg.); this suggests that the sympathomimetic response in Rana pipiens is primarily due to the excitatory action of epinephrine rather than levo-arterenol.

Although Robbins and Parker (1949) noted that hypophysectomy failed to alter the sensitivity of the male Xenopus laevis and Rana pipiens to epinephrine the possible role of the pituitary as an adrenergic link in the gonadotropic induced emission of spermatozoa has not been established. The trigger mechanism is probably the excitation of an "alpha adreno-

tropic receptor" (Ahlquist, 1948) in the sperm ejaculatory apparatus of the frog; gonadotropins may possibly cause the release of endogenous epinephrine in the frog or may block a cholinergic receptor.

SUMMARY AND CONCLUSIONS

In rather extensive studies on over 500 frogs we have found that several representative adrenolytic drugs will block the release of spermatozoa by the adult male frog, Rana pipiens, if injected in the dorsal lymph sac prior (30 seconds to one minute) to the injection of chorionic gonadotropin, pituitary gonadotropin, human pregnancy urine, levo-epinephrine, levo-arterenol and epinephrine hydrochloride.

The optimal dose of adrenolytic agent necessary for inhibition of emission of spermatozoa was found to be:

Benodaine Hydrochloride—0.5 to 2.5 mg.
Beta-chlorethylamine S.K.F. 688-A—2.0 to 3.0 mg.
Compound C.C.K. 179—1.0–4.0 mg.
(a mixture of the alkaloids of ergotoxine)
Priscoline Hydrochloride—10.0 mg.
Dibenamine Hydrochloride—2.0–12.5 mg.

Studies thus far suggest that Benodaine Hydrochloride is the most effective agent in inhibiting release of spermatozoa in the male frog.

Doses of the control gonadotropic substances were as follows: 50 I.U. chorionic gonadotropin; 1 R.U. pituitary gonadotropin; and 1 cc. to 5 cc. unconcentrated human pregnancy urine. These dosages were above the minimal dose level required to produce a positive pregnancy test in Rana pipiens.

The approximate minimal dose of levo-epinephrine producing gametokinesis was 0.005 mg., and of levo-arterenol, 0.05 mg. Dosages of epinephrine hydrochloride injected varied from 0.01 to 0.02 mg. as smaller quantities did not produce a uniformly positive response (gametokinesis).

Whether such substances as atropine and histamine will inhibit the gonadotropic or adrenergic induced release of spermatozoa has not been studied.

Evidence obtained strongly suggests that the emission of spermatozoa is mediated by the epinephrine activation of an adrenotropic receptor in the sperm ejaculatory apparatus of the male frog. The possible role of the pituitary in provoking endogenous epinephrine release and subsequent emission of spermatozoa upon the injection of pituitary and chorionic gonadotropins has not been established. Rana pipiens promises to be a good test animal for the bioassay of epinephrine by standardizing (1) the dose of epinephrine necessary for gametokinesis and (2) the dose of adrenolytic drug necessary to inhibit epinephrine-induced gametokinesis.

REFERENCES

AHLQUIST, R. P.: *Am. J. Physiol.* **153:** 586. 1948.
GREENBLATT, R. B., S. L. CLARK, AND R. M. WEST: *Am. J. Physiol.* **159:** 571. 1949.
GREENBLATT, R. B., S. L. CLARK AND R. M. WEST: *J. Clin. Endocrinol.* **10:** 265, 1950.
GREENBLATT, R. B., S. L. CLARK AND R. M. WEST: Fertility and Sterility **1:** 533. 1950.
MILLER, D. F. AND P. B. WILTBERGER: *Ohio J. Science* **48:** 89. 1948.
ROBBINS, S. L. AND F. PARKER, JR.: *Endocrinology* **42:** 237. 1948.
ROBBINS, S. L. AND F. PARKER, JR.: *Endocrinology* **44:** 384. 1949.
SOUCY, L. B.: *Am. J. Med. Tech.* **16:** 6. 1950.
WALDROP, E. G. AND M. F. JAMES: *South. Med. J.* **43:** 886. 1950.
WILTBERGER, P. B. AND D. F. MILLER: *Science* **107:** 198. 1948.

GONADAL INFLUENCES ON SURVIVAL AFTER ADRENALECTOMY IN THE GOLDEN HAMSTER

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RELATIVELY few reports have appeared on the endocrine glands of the golden hamster (*Mesocricetus auratus*). Peczenik (1944) demonstrated a sexual dimorphism in the size of the adrenal gland, that of the male being larger than that of the female. This condition is opposite to that in the rat and indeed, to that found in most other mammals investigated (Hill 1930; Parkes 1945). Gonadectomy equalizes the size of the male and female adrenals (Peczenik 1944; Keyes 1949) as it does in the rat (Parkes 1945). The equalization is effected by enlargement of the female gland and a decrease of the male gland. This effect can be reversed in the male by treatment with androgen, but not in the female with estrogens (Peczenik 1944). The size of the adrenal of the female rat is reduced by administration of androgen (Hall 1940), and Parkes (1945) concluded that the male adrenal is smaller than that of the female because of suppression exerted by testicular activity. In the hamster, however, androgen appears to have either a direct or an indirect stimulating effect on the size of the adrenal. Various investigators have shown that adrenocortical and ovarian hormones can partly replace one another in therapy following adrenalectomy. Injection of gonadotropins or progesterone increases the survival time of adrenalectomized rats (Greene, Wells and Ivy 1939; Schwabe and Emery 1939; Bourne 1939), and desoxycorticosterone acetate has a progestational effect in the hamster (Isaacson 1949). Several workers have pointed out changes in the adrenal cortex associated with the estrus cycle of the rat (Anderson and Kennedy 1932) and hamster (Peczenik 1944), and others have reported alterations in the estrus cycle of rats in adrenal insufficiency (Wyman 1927; Corey and Britton 1934). There is a difference of opinion as to whether these effects are the result of direct interaction between the adrenal and the ovary or of mediation by the pituitary-end organ axis. Experiments were undertaken to discover if the sexual dimorphism in the adrenals of the hamster is reflected in a physiological sex difference, and to attempt a clarification of the differences between the hamster and the rat.

MATERIALS AND METHODS

For a study of survival, 78 male hamsters from 8 to 25 weeks of age and 52 females from 8 to 20 weeks of age were bilaterally adrenalectomized through a dorsolateral ap-

Received for publication April 23, 1951.

proach in a single operation under ether anesthesia. Fifteen additional females in the same age range were ovariectomized and 24 were ovariectomized and then adrenalectomized, in a single operation through the same incision. Twelve other females were ovariectomized and 3 each were adrenalectomized 7, 14, 21, and 30 days after the first operation. All animals were fed Purina Laboratory Chow *ad libitum* and tap water. No special post-operative care was taken except that the animal room was never below 70 degrees F. Body weights were recorded at least every other day and vaginal smears were taken daily from all females, beginning one week prior to operation. Only females with regular estrus cycles were used in the experiments. Five females, 20 weeks old, were adrenalectomized and vaginal smears were omitted from the post-operative routine to check the possibility of cervical stimulation causing pseudo-pregnancy and a consequent alteration of survival time. Ten males were subjected to blank operations as controls for the surgical procedures.

Ovaries of normal female hamsters of all age groups and in all stages of estrus were sectioned and examined to determine the relative number of corpora lutea.

To test the effect of steroids on survival desoxycorticosterone acetate (DOCA), desoxycorticosterone glucoside (DOCG), or progesterone were injected subcutaneously. In a preliminary series it was found that a daily injection of at least 0.5 mg. of progesterone or 1.0 mg. of DOCA or DOCG was necessary to maintain the body weight of adrenalectomized male or adrenalectomized-ovariectomized female hamsters, so in subsequent experiments daily injections of 0.8 mg. of progesterone or 2.5 mg. of the adrenal steroids were given. All of the females used were from 8 to 20 weeks of age and were adrenalectomized and ovariectomized to remove the variables of ovarian origin. Except for two groups of females noted below, 7 daily injections of the steroid were given, beginning immediately after the operation and ending on the sixth post-operative day. Twenty-one females and 5 males were thus treated with progesterone. Two other females were given daily injections for 30 days. Fourteen females and 7 males were treated for 7 days with DOCA, and 3 other females were treated for 30 days. Seven females were treated for 7 days with DOCG.

RESULTS

Survival time of males. The range of survival of adrenalectomized male hamsters was from the fourth to the eleventh post-operative day (Fig. 1 A) with a mean of 6.7 days (σ 1.6). There was constant and rapid weight loss (Fig. 1 C), totaling 26 per cent of the weight at the time of operation. The blank operated control males survived indefinitely and their body weights were not affected significantly.

Survival time of females. The range of survival of all adrenalectomized female hamsters was from 5 to 17 days after the operation (Fig. 1 B), with a mean of 10.2 days (σ 3.1). The difference between the survival times of males and females is significant (Fisher's table for t). The mean total weight loss was 28 per cent (Fig. 1 C).

Adrenalectomized-ovariectomized females. The range, 5 to 11 days, the mean of 7.0 days, and the form of the graph (Fig. 1 A) were almost identical to those of the males. The mean total weight loss was 35 per cent (Fig. 1 C). Ovariectomy alone inhibited growth, the body weight remaining fairly constant for 30 days after operation, but it did not threaten survival.

Adrenalectomy and estrus. Adrenalectomy either had no effect at all on the estrus cycle, or there was extension of metestrus. A duration of more than two days was considered as extension (after Ward 1946). Of 26 females, 12 weeks old or younger, 9 or 34.6 per cent exhibited extended metestrus (4 to 12 days) after adrenalectomy. Of 24 animals, 14 weeks old or older, 15 or 62.5 per cent displayed extended metestrus (5 to 13 days). Examination of the ovaries of normal females in metestrus confirmed the

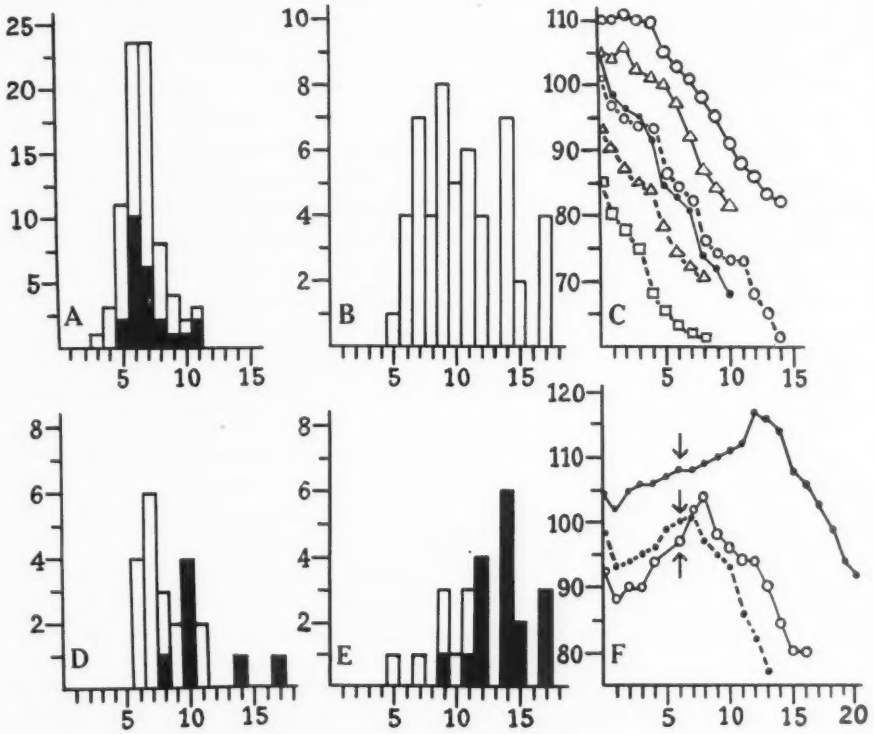

FIG. 1. Survival times (A, B, D, E) and mean body weights (C, F) of adrenalectomized hamsters. Abscissas represent days after adrenalectomy; ordinates in A, B, D, and E represent number of animals, in C and F body weight in grams. A: 78 males, and 24 ovariectomized and adrenalectomized females (solid black). B: 52 females. C: broken lines, males (squares) and females under 14 weeks old; solid lines, ovariectomized and adrenalectomized females (solid dots) and females over 14 weeks old; triangles, normal estrus cycles; circles, extended metestrus. D: 24 females 13 weeks old or younger; solid black, extended metestrus. E: 26 females 14 weeks old or older; solid black, extended metestrus. F: ovariectomized and adrenalectomized hamsters treated with progesterone (solid line, circles), DOCG (broken line), or DOCA (solid line, solid dots); last injections at arrows.

observations of Ward (1946) that during vaginal metestrus the corpora lutea are well established.

Age of females and survival. There are three peaks, at 7, 9, and 14 days after operation, in the graph of survival times of adrenalectomized female hamsters (Fig. 1 B). Analysis of this group revealed that animals under 14 weeks of age with normal estrus cycles survived from 6 to 11 days after adrenalectomy (Fig. 1 D), with a mean of 7.6 days (σ 1.5), while those with extended metestrus survived from 8 to 17 days, with a mean of 10.8 days (σ 2.9). The difference between these two groups is significant but only the mean survival time of those with extended metestrus is significantly different from that of the males. Female hamsters 14 or more weeks of age with normal estrus cycles survived 5 to 11 days (Fig. 1 E), with a mean of 9.1 days (σ 1.7), but those with extended metestrus survived 9 to 17 days, with a mean of 13.4 days (σ 2.1). These two groups differed significantly from each other and from the males. The mean survival time of the 5 female hamsters which were not subjected to vaginal smears was 13.8 days.

Effect of steroids. Adrenalectomized-ovariectomized female hamsters treated with progesterone for 7 days after operation survived from 14 to 18 days, and their body weight increased until the second day after the last injection, when it began to decrease progressively until death of the animal (Fig. 1 F). Those treated for 30 days had a progressive increase in body weight. Adrenalectomized males behaved similarly. Apparently exogenous progesterone will maintain adrenalectomized hamsters, with a normal growth curve, indefinitely.

Female hamsters treated with DOCA for 7 days after operation survived from 17 to 20 days and gained weight until 6 days after the last injection (Fig. 1 F). The three animals treated for 30 days gained considerable weight (40 gm.). Adrenalectomized males treated with DOCA behaved similarly.

Female hamsters treated with DOCG for 7 days after operation survived from 11 to 13 days, but the mean body weight reached a level of only 2 gm. above the original weight, on the day after the last injection, and then declined progressively until death. DOCA appears to be about twice as effective as the water soluble glucoside in maintaining the adrenalectomized hamster. The increase in body weight and the length of survival was greater in the DOCA treated animals than in those receiving DOCG, and the beneficial effect of DOCA lasted until 6 days after the last injection, while that of DOCG lasted only one day. This confirms Cosmos, Duell, and Gaunt (1950) who found that DOCA in oil is about five times as effective as this glucoside in maintaining life and growth in adrenalectomized rats.

Progesterone, on the other hand, seems to be more effective than either. The absolute amount of progesterone needed to maintain the adrenalectomized hamster (0.5 mg.) was one half the minimum amount of DOCA required (1.0 mg.), and the effectiveness of a daily dose of progesterone (0.8 mg.) which was about one third that used of DOCA or DOCG (2.5 mg.) was only slightly less than that of the DOCA and greater than that of the DOCG.

DISCUSSION

Cowie (1949) found that mature female rats lived longer after adrenalectomy than males and attributed the difference to the secretion of progesterone in the females. He also found that the survival time of both sexes increased with the age at operation. Our experiments showed that the age of male hamsters cannot be correlated with the survival time after adrenalectomy but that sex is highly significant, females surviving longer than males. Moreover ovariectomy equalized the survival times of the sexes. Apparently some secretion of the ovary protects the females. The protection is greater in females over 14 weeks of age than in younger animals, and greater in those of both age groups that have extended metestrus after adrenalectomy than in those with normal cycles. Rolle and Charipper (1949) have found that there is a sudden increase in the average number of corpora lutea in the ovaries of hamsters between the third and fourth months of age, and our own observations have confirmed this. This increase occurs at about the same time as the increase in survival time after adrenalectomy. Apparently there is an increased receptivity of ovarian tissues to gonadotropic stimulation at this time. Moreover, an increased number of corpora lutea was found during metestrus, so it is likely that the factor which is responsible for longer survival in females than in males, or in older females and in those with extended metestrus, is an increased secretion of progesterone. This is supported by the fact that progesterone will maintain an adrenalectomized hamster. Apparently the smaller number of corpora lutea in the ovaries of hamsters under 14 weeks of age in which metestrus is not extended, do not secrete enough progesterone to extend survival time after adrenalectomy beyond that of males. The difference between hamsters and rats may be attributed to a greater sensitivity of the tissues of hamsters to the action of progesterone. The absolute amount of DOCA necessary to keep the rat and the hamster alive after adrenalectomy appears to be about the same, but the absolute amount of progesterone needed to protect the hamster is one half the smallest dosage which benefits the rat. Emery and Greco (1940) suggested that there is a one to one relationship between DOCA and progesterone in their effects in the rat, but in the hamster it appears to be two to one.

The extension of metestrus after adrenalectomy may depend upon the removal of one member of a synergistic or cooperative pair of factors which determine its length in the intact animal. In the normal estrus cycle, metestrus may be terminated when the secretion of progesterone reaches a level high enough to inhibit the secretion of luteinizing or luteotrophic hormones from the anterior pituitary and this inhibitory action may be aided or hastened by a similarly inhibitory action of adrenocortical hormone (ACH). Peczenik (1944) observed that the size of the adrenal cortex of the female hamster varies throughout the estrus cycle, and is greatest, about equal to that of the male, during metestrus. After adrenalectomy,

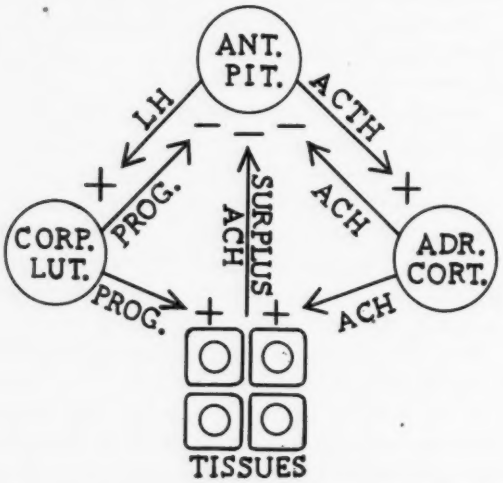

FIG. 2. Reciprocal relationships between the anterior pituitary, adrenal cortex, corpora lutea, and tissue cells.

this auxiliary action of ACH is missing and metestrus is extended. The possibility that cervical stimulation, resulting from taking vaginal smears, altered ovarian activity so as to increase survival was ruled out by the experiments in which survival time was lengthened when no vaginal smears were taken.

The greater incidence of extension of metestrus in the older females may result from the increase in sensitivity of ovarian tissues with advancing age mentioned above. Any imbalance in the hormonal system would be more likely to affect the more receptive tissues. The reason why some female hamsters do not exhibit extended metestrus after adrenalectomy is more obscure, but it may depend upon considerable individual variation in the development of increased receptivity.

The sexual dimorphism in the size of the adrenal gland of the hamster may not be entirely the result of some stimulating effect of androgen. The size of the adrenal gland may be regulated by the output of adrenocorticotropic hormone (ACTH) from the anterior pituitary, which in turn is regulated by a reciprocal inhibitory action of ACH, the titer of which in the blood is determined by the demand of tissue cells for adrenocortical support (Sayers 1950). Since hamsters seem to be more responsive to progesterone than do rats, and since progesterone seems to exert a beneficial action similar to that of ACH, it may be that ovarian progesterone joins with ACH in lessening the demand of the tissues (Fig. 2). Hence the titer of ACH in the blood would rise and ACTH output would be inhibited. Moreover, progesterone may inhibit ACTH output directly as does ACH. Both actions would contribute to the small size of the adrenal gland in the female and account for increase in size after ovariectomy. In rats the dimorphism may be reversed because androgen suppresses adrenal size in that species and the tissues are not so responsive to progesterone. Two of Peczenik's findings (1944) are contradictory to this theory, the increased size of the adrenal cortex of the female hamster during metestrus when progesterone secretion should be at its height, and the failure of ovarian hormones to reverse tne castration effect. The first observation, however, may be the result of a time lag in adjustment of adrenal size during the estrus cycle, the larger size resulting from a low level of progesterone secretion before metestrus which carries over into metestrus. The second finding may have resulted from the use of estrogens rather than progesterone. Obviously the influence of injections of the latter hormone on adrenal size should be investigated.

SUMMARY AND CONCLUSIONS

The post-operative survival time of 78 adrenalectomized male hamsters averaged 6.7 days, that of 52 females 10.2 days. Both lost about one quarter of their body weight. Ovariectomy equalized the survival times of the sexes. Extension of metestrus followed adrenalectomy in 34.6 per cent of females under 14 weeks of age, and in 62.5 per cent of older animals, and survival times averaged longer in these (10.8 and 13.4 days) than in females with normal cycles (7.6 and 9.1 days). Exogenous DOCA, DOCG, or progesterone maintained life and body weight in adrenalectomized hamsters, the necessary amount of progesterone being half that of DOCA or DOCG. It is concluded that ovarian progesterone prolongs the life of adrenalectomized female hamsters, and that the differences with age or with alteration of estrus cycles result from different numbers of active corpora lutea. It is proposed that sexual dimorphism, and age and species differences in adrenal morphology and physiology depend upon differences

in the hormonal balance within the pituitary-adrenal-ovarian-tissue-cell axis acting upon tissues varying in their sensitivity to hormonal stimulation.

Acknowledgments

We are indebted to Dr. Frederick F. Yonkman, Ciba Pharmaceutical Products, Summit, New Jersey, for generous supplies of desoxycorticosterone acetate (Percorten), Percorten Glucoside, and progesterone (Lutocylin).

REFERENCES

ANDERSON, D. H. AND H. S. KENNEDY: *J. Physiol.* **76:** 247. 1932.
BOURNE, G.: *J. Physiol.* **95:** 12P. 1939.
COREY, E. L. AND S. W. BRITTON: *Am. J. Physiol.* **107:** 207. 1934.
COSMOS, E., H. DUELL AND R. GAUNT: *Endocrinology* **46:** 30. 1950.
COWIE, A. T.: *J. Endocrinol.* **6:** 94. 1949.
EMERY, F. E. AND P. A. GRECO: *Endocrinology* **27:** 473. 1950.
GREENE, R. R., J. A. WELLS AND A. C. IVY: *Proc. Soc. Exper. Biol. & Med.* **40:** 83. 1939.
HALL, K.: *J. Path. & Bact.* **51:** 75. 1940.
HILL, W. C. O.: *J. Anat.* **64:** 479. 1930.
ISAACSON, J. E., JR.: *Endocrinology* **45:** 558. 1949.
KEYES, P. H.: *Endocrinology* **44:** 274. 1949.
PARKES, A. S.: *Physiol. Reviews* **25:** 203. 1945.
PECZENIK, O.: *Proc. Soc. Roy. Soc. Edinburgh* **62:** 59. 1944.
ROLLE, G. K. AND H. A. CHARIPPER: *Anat. Rec.* **105:** 281. 1949.
SAYERS, G.: *Physiol. Reviews* **30:** 241. 1950.
SCHWABE, E. L. AND F. E. EMERY: *Proc. Soc. Exper. Biol. & Med.* **40:** 383. 1939.
WARD, M. C.: *Anat. Rec.* **94:** 139. 1946.
WYMAN, L. C.: *Am. J. Physiol.* **86:** 528. 1928.

THE EFFECTS OF HYPOPHYSEAL GROWTH HORMONE ON THE METABOLISM OF Ca^{45} IN HYPOPHYSECTOMIZED RATS*

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ALTHOUGH the effects of hypophyseal growth hormone on bone histology have been thoroughly studied, little experimental work has been done on the interrelationships of this hormone and calcium metabolism. The few reports appearing in the literature have been inconclusive. Teel and Cushing (1930) noted that the urinary excretion of calcium in dogs was increased during the first few days following the injection of growth stimulating extracts. Pugsley and Anderson (1934) found that hypophysectomized rats on a low calcium diet showed a negative calcium balance which became positive on the administration of a crude growth hormone preparation. Krishnan (1942), using rats and guinea pigs, showed that injections of growth-promoting extracts caused calcium retention in these animals. It was with the object of studying the effects of pure growth hormone administration on the metabolism of Ca^{45} in hypophysectomized rats that the following experiment was performed.

METHODS

Sprague-Dawley male rats were hypophysectomized at 64 days of age. 69 days after operation, 5 of these animals (onset controls) were briefly anesthetized with ether and injected intravenously by way of the external jugular vein with 0.2 cc. of $Ca^{45}Cl_2$ per 100 grams of body weight. The solution, which was dissolved in 0.9 per cent NaCl, contained approximately 8 micrograms of calcium tagged with 4 microcuries of Ca^{45}.

After injection, each animal was placed in a glass beaker which was provided with a wire screen for the separation of urine and feces; two hours later the animal was sacrificed with chloroform. Blood was obtained by puncture from the exposed heart. An aliquot of serum was placed on an aluminum disc, air dried, and the amount of radioactive calcium in the sample determined by counting it with a thin window Geiger-Mueller counter. Suitable self-absorption corrections were made on all samples. The total amount of serum in the animal was calculated by using the value of 2.54 cc. of serum per 100 grams of body weight 66 days after hypophysectomy, as given by Berlin and co-workers (1950).

Both tibias were removed; one was frozen at −20° C. for radio autographic studies.

Received for publication April 23, 1951.

* Aided in part by a grant from the National Institutes of Health of the United States Public Health Service.

The other was cleaned and placed in a tared crucible, dried, ashed, dissolved in 1 *N* HCl and made up to volume with distilled water. In order to determine its Ca^{45} content, an aliquot of the dissolved tibia was treated in a manner similar to that of the serum. A modification of Larson and Greenberg's method (1938) was used for determining the total calcium content (isotopic plus non-isotopic) of each tibia.

To collect the urine, the wire screen and beaker were rinsed with 1 *N* HCl and distilled water. The contents of the bladder were added to this and the resulting washings transferred to a volumetric flask. The feces, together with the contents of the large intestine, were placed in a crucible and ashed. The Ca^{45} content of the urine and feces was then determined.

On the day that the onset controls were sacrificed, seven animals began receiving intraperitoneally a single daily dose of 0.2 mg. of pure growth hormone. The daily dose was increased to 0.5 mg. on the eighth day after the injections were begun. Thus at the end of the three-week injection period the treated animals had received a total of 8.4 mg. of growth hormone. The hormone was isolated from beef anterior pituitary glands by the method of Li *et al.* (1945). Seven other animals, injected intraperitoneally with 0.9 per cent NaCl, served as saline controls. All animals were fed ad libitum.

After 21 days of treatment both groups of animals were injected with the same amount of radiocalcium as was given to the onset controls. Exactly the same procedures were used as for the onset group. The total amount of serum in these two groups was calculated by using Berlin's value (1950) of 2.83 cc. of serum per 100 grams of body weight, 98 days after hypophysectomy.

The technique used in this experiment for obtaining radioautographs of the split tibias has been described previously by Ulrich *et al.* (1951).

RESULTS

Table 1 presents a summary of the data obtained. The figures for the per cent dose represent the amount of Ca^{45} in the tissue per 0.2 cc. of $Ca^{45}Cl_2$ injected. The specific activity of the tibia represents the per cent of the injected dose, per milligram per cent of calcium in the bone ash.

TABLE 1. THE EFFECTS OF GROWTH HORMONE ON THE METABOLISM OF Ca^{45} IN HYPOPHYSECTOMIZED RATS

Group	No. of rats	Average body weight			Serum Per cent dose	Tibia			Urine Per cent dose	Feces Per cent dose
		Onset	Autopsy	Net gain		Per cent dose	Total Ca in ash	Specific activity		
		gm.	gm.	gm.			mg. %			
Onset controls	5	176	176	—	5.56±0.23[1]	2.35±0.12	38.5±1.0	0.06	0.37±0.10	1.29±0.19
Saline controls	7	184	174	−10	5.60±0.33 (<0.9)[2]	1.96±0.14 (<0.4)	39.6±0.4	0.050 (<0.001)	1.16±0.14 (<0.001)	0.99±0.06 (<0.1)
Hormone treated	7	182	235	+53	4.81±0.22 (<0.1)	4.55±0.23 (<0.001)	39.0±0.6	0.117	1.28±0.23 (<0.6)	1.10±0.15 (<0.5)

[1] Mean ± standard error.
[2] Fisher's P values. The saline controls are compared to the onset controls, and the growth hormone treated are compared to the saline controls.

The level of radiocalcium in the serum is not significantly different in the three groups. More than twice as much Ca^{45} was found in the tibias of the treated animals as in the onset and the saline controls; the same proportionate difference was also observed in the specific activity. The calcium content of the tibias expressed as milligrams per 100 milligrams

of bone ash, was similar in all three groups ranging in value from 38.5 to 39.6 mg. per cent.

The urinary excretion of Ca^{45} during the 2 hour period was very low in all groups, although lower in the onset controls than in the other two groups. Fecal excretion was also very small and similar in all three groups.

Radioautographs of the split tibias showed a dense line of deposited Ca^{45} in the proximal epiphyseal plate region of the treated animals which was not seen in the saline controls or the onset controls (see Figure 1).

At the end of the injection period the treated animals had gained an average of 53 grams (range: +35 to +72 grams) while the saline controls

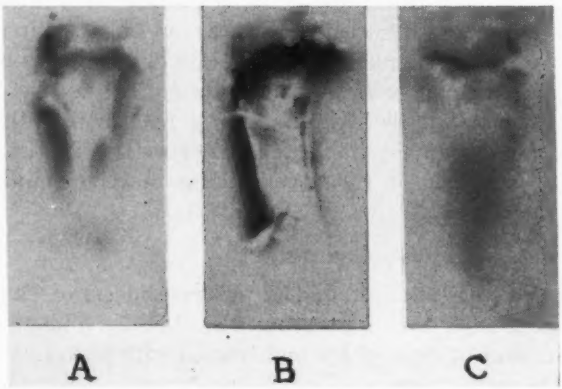

FIG. 1. Radioautographs of split tibias of hypophysectomized rats two hours after the intravenous injection of a tracer dose of Ca^{45}. A. Onset control; B. Growth hormone treated; C. Saline control.

had lost 10 grams (range: −4 to −20 grams). These changes correspond to a 25 per cent weight increase by the growth hormone treated animals as compared with their controls.

DISCUSSION

Marx and Reinhardt (1942) using radiostrontium found no difference in the deposition of this element in the bones of hypophysectomized growth hormone treated rats and hypophysectomized controls. The findings reported in this study using radioactive calciu n do not confirm their results. It should be pointed out, however, that the experimental procedures differed in the two experiments. Marx and Reinhardt used young animals which received total doses of growth hormone varying in amount from 0.6 to 3.2 mg. over short periods. The animals used in this experiment were mature plateaued rats which received a total of 8.4 mg. of growth hormone over a 21-day period. Furthermore, some of the animals used in the Marx

and Reinhardt experiment were injected more than once with radiostrontium, whereas only one injection of Ca^{45} was given to the animals reported in this study.

The heavy deposit of Ca^{45} in the proximal epiphyseal plate region as seen by radioautography in the hormone treated animals may account for the greater Ca^{45} content in the tibias of these animals and may be associated with the renewed growth activity. In a previous paper, Ulrich *et al.* (1951) demonstrated the appearance of an intense line of deposited Ca^{45} in the proximal epiphyseal plate region of tibias from 60-day old non-treated hypophysectomized rats which corresponded to the site of the sealing off bone that has been shown to develop at about this time in these animals (Becks, Simpson and Evans, 1945). This was not observed in our preparations where the animals were 133 and 154 days old at the time of autopsy. Since the epiphyseal plate region is the principal site for the longitudinal growth of long bones, growth hormone, by reactivating this region in hypophysectomized animals, would tend to cause the uptake of a greater amount of Ca^{45} here than in the corresponding inactive epiphyseal region of the non-treated controls.

SUMMARY

1. The effects of growth hormone on the metabolism of Ca^{45}, two hours after intravenous injection, were studied in 154-day old male rats, hypophysectomized at 64 days of age and treated with pure growth hormone from 133 to 154 days of age.

2. There was no difference in the Ca^{45} in the serum of the onset controls, the saline controls and the treated animals.

3. The tibias of the treated animals took up twice as much Ca^{45} as did those of the other two groups, although the calcium content of the ash was the same in all three groups.

4. Radioautographs of the split tibias showed an intense line of Ca^{45} deposited in the proximal epiphyseal plate region of the growth hormone treated animals.

5. No significant differences were found in the urinary and fecal excretion of Ca^{45} between the treated and the two control groups.

The authors wish to acknowledge the advice and suggestions of Dr. D. Harold Copp.

REFERENCES

Becks, H., M. E. Simpson and H. M. Evans: *Anat. Rec.* **92**: 121. 1945.

Berlin, N. I., D. C. Van Dyke, W. E. Siri and C. P. Williams: *Endocrinology* **47**: 429. 1950.

Krishnan, K. N.: *Indian J. Med. Research* **30**: 589. 1942.

LARSON, C. E. AND D. M. GREENBERG: *J. Biol. Chem.* **123:** 199. 1938.
LI, C. H., H. M. EVANS AND M. E. SIMPSON: *J. Biol. Chem.* **159:** 353. 1945.
MARX, W. AND W. O. REINHARDT: *Proc. Soc. Exper. Biol. and Med.* **51:** 112. 1942.
PUGSLEY, L. I. AND E. M. ANDERSON: *Am. J. Physiology* **109:** 85. 1934.
TEEL, H. M. AND H. CUSHING: *Endocrinology* **14:** 157. 1930.
ULRICH, F., D. H. COPP, C. W. ASLING, C. H. LI AND W. O. REINHARDT: *Endocrinology* **48:** 245. 1951.

SYSTEMIC LEVELS OF PLASMA PROGESTERONE DURING PREGNANCY IN WOMEN AND MONKEYS[1]

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SEVERAL attempts have been made to measure the progestin content of blood samples obtained during pregnancy. In 1933, Clauberg, Thiel, and Ziecker failed to detect by bio-assay any corpus luteum hormone in ether extracts of 12–50 cc. of serum and of 300 cc. of blood from pregnant women. Similarly, Bloch (1936), employing the Corner-Allen test, found no activity in up to 500 cc. of blood from pregnant women or 70 cc. of blood from pregnant rabbits; even 8 to 12 liters of sow pregnancy blood yielded the equivalent of less than one rabbit unit. More recently, Hoffmann and von Lam (1941, 1942, 1948) and Haskins (1941) conducted bioassays based on the reaction of the endometrium of the rabbit to the intrauterine injection of blood extracts; these investigators found progestin in human pregnancy blood, but the assay results were variable.

Following the development of a bio-assay method which appears to be sensitive, accurate, and at least reasonably specific for progesterone (Hooker and Forbes, 1947, 1949a), a further study of plasma levels of this hormone in pregnant women and monkeys was undertaken. Possible discrepancies between values obtained by this method and by the physicochemical methods of others (Haskins, 1950; Morris, personal communication) have raised a question as to whether the Hooker-Forbes bioassay should be regarded as specific for progesterone or only for progestin. In this report the term *progesterone* will be used, but with some reservation.

MATERIALS AND METHODS

The various stages of pregnancy in the two human donors were timed in relation to the beginning of the last menstrual period. The dates of conception were known for the

Received for publication April 23, 1951.

[1] This investigation was aided by grants from the Committee for Research in Problems of Sex, National Research Council, from the National Institutes of Health, Public Health Service, and from the James Hudson Brown Memorial Fund of the Yale University School of Medicine.

The author is deeply indebted to the donors, to Drs. J. T. Wolstenholme, S. L. Palay, and Martin Wolfish, who drew the human blood samples, and to Dr. Gertrude van Wagenen, Department of Obstetrics and Gynecology, Yale University, and her assistants for obtaining the numerous blood samples from monkeys in Dr. van Wagenen's colony.

three monkey donors, since during her last menstrual cycle each animal had been segregated from the male except for one day.

Blood samples (approximately 1 cc.), obtained from arm veins of the women and from leg veins of the monkeys, were promptly centrifuged with 0.05 cc. of 20% sodium citrate solution as anticoagulant. The plasma and anticoagulant were then removed, measured, and precipitated in cold acetone. The resulting mixture was stored at about 4° C. until it could be assayed for "free" and "bound" progesterone according to a standard procedure (Hooker and Forbes, 1949a).

The blood samples were usually taken at intervals of a week, but they were obtained more frequently from the monkeys during the last weeks before delivery.

One human and one monkey donor were delivered by Caesarian section. Except for the birth of female twins to one monkey (B3), all births were single. The pregnancies appeared to be normal, the babies were healthy, and the mothers made uneventful recoveries.

RESULTS

The results of the assays for free and bound progesterone were plotted against the duration of pregnancy (Figures 1 and 2). On the charts, the symbols consisting of a circle and an arrow signify that less than the indicated amount of hormone was present; the assay does not reveal the absence of progesterone. The closed circles, indicating individual assay values for the free hormone, are connected by lines in order to facilitate visualization, but it should be clearly understood that this is not meant to imply that intermediate concentrations of the hormone necessarily would fall on the lines.

Bound progesterone. The level of bound hormone was always relatively low (i.e., 0.5 μg./cc. plasma, or less); frequently, no bound hormone could be detected.

Free Progesterone before Delivery. The levels of the free hormone did not

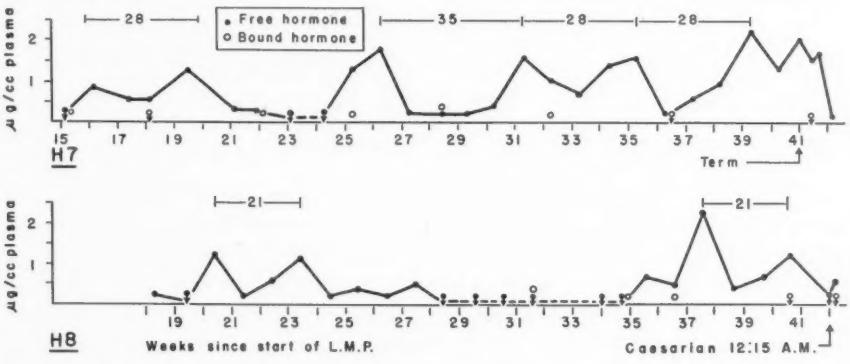

FIG. 1. Time is indicated on the abscissae in weeks since the beginning of the last menstrual period (L.M.P.) Intervals between some of the peaks of free hormone concentration are indicated on an upper scale.

exceed about 2 μg./cc. plasma in the women and about 3 μg./cc. in the monkeys. During each pregnancy there was at least one instance when no hormone could be found in from one to seven successive weekly samples. Such low periods were usually followed by rather abruptly rising peaks.

While there appears to be no regularity in the occurrence of intervals when no progesterone could be detected, there is some suggestion of regularity in the temporal spacing of the peaks of plasma concentration of

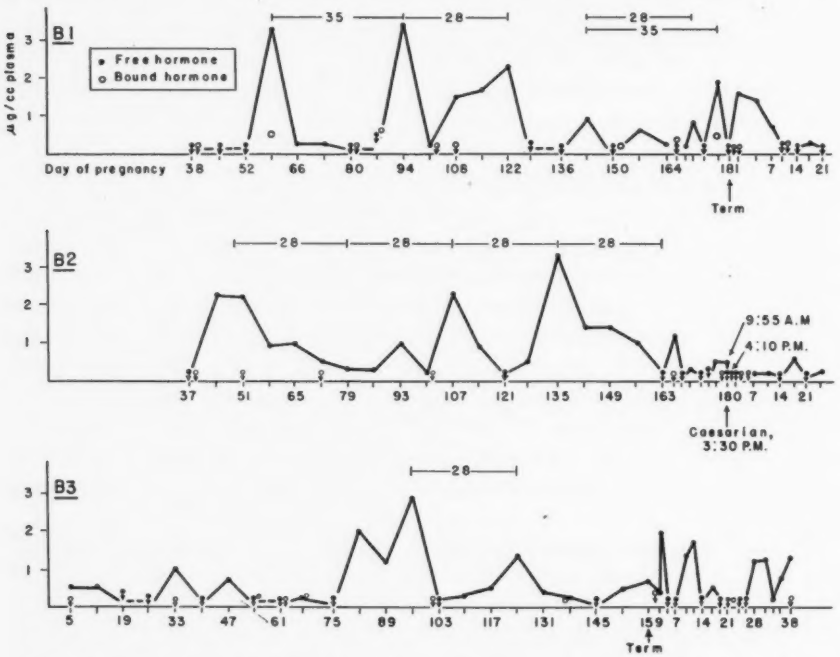

FIG. 2. Time is indicated on the abscissae, first on the basis of days of pregnancy and then of days after delivery. As in Figure 1, a pattern for the time intervals between some of the peaks of plasma concentration is suggested.

the hormone (Figures 1 and 2). Because blood samples were usually taken at weekly intervals, such peaks as are revealed occur at multiples of seven days. As indicated on the charts, the peaks usually fit a four-week cycle, although some peaks seem to recur on a three-week or five-week pattern. Also, it is likely that some elevations of the plasma levels of the hormone occurred between the times when blood samples were drawn; if so, these peaks of course were undetected.

Free Progesterone at Term. In all three monkeys the level of free hormone was low at the time of delivery; in B2 and B3 the level had been low for

several days before delivery. Monkey B2 was delivered by Caesarian section because labor was judged to be overdue and because of fear of impending toxemia.

In human donor H7 the concentration of the hormone was 2.0 μg./cc. four hours before a normal, spontaneous delivery. In H8, however, a very different situation existed. A blood sample subsequently found to contain less than 0.25 μg./cc. plasma was drawn at 10:30 P.M. on June 5; at this time the patient had been in labor for about 49 hours. The membranes had been ruptured artificially approximately 14 hours after labor had begun. There was no pelvic disproportion. Because of uterine inertia, a Caesarian section was ultimately performed, and a normal baby was delivered at 12:15 A.M. on June 6. An additional blood sample was drawn about 7:00 A.M. the same day; by this time the level of free progesterone had risen to 0.6 μg./cc.

A sample of umbilical cord blood had been obtained from H8 at 12:17 A.M. There was a delay of about an hour before this sample was refrigerated, and hemolysis occurred. Assay showed that the sample contained 1.4 μg. free progesterone/cc. whole blood.

Free Progesterone after Delivery. Plasma progesterone concentrations in H7 were 1.5 μg./cc. on the third day after delivery, 1.7 μg./cc. on the fifth day, and 0.2 μg./cc. on the eighth day (Fig. 1). Lactation began promptly, became copious, and continued for about five months.

Plasma progesterone concentrations in monkeys B1 and B3 fluctuated sharply after birth of the young. In B2 the level of the hormone was persistently low during the same period. It is perhaps significant that, disregarding for the moment the time of delivery, all three animals showed peaks of concentration of the hormone at 166–178 days after conception (in B1, on days 171 and 178, in B2 on day 166, and in B3 on days 164, 168, and 171 after conception).

The offspring of B1 and B2 were taken from them, partly because of maternal neglect. There is no record as to whether the mothers showed signs of lactation. B3 was able to nurse one of her twins; the other was fed by bottle.

DISCUSSION

The observation that the "bound" fraction of the plasma progesterone is low is consistent with studies of bound progesterone levels in the plasma or blood of intact, untreated mice, rabbits, and monkeys (Hooker and Forbes, 1949a and unpublished data; Forbes, Hooker, and Pfeiffer, 1950a, b; Forbes, unpublished data) and in the plasma of normal, untreated women (Forbes, 1950). These observations were made during various stages of the estrous and menstrual cycles, in pseudopregnancy, and in

pregnancy. Frequently the assay failed to detect any bound progesterone.

Haskins, using an intra-uterine bio-assay, detected in ether extracts of serum from human pregnancy blood a quantity of progestationally active material equivalent to less than 0.13 μg. progesterone/cc. serum (1941). In a later experiment (1950) he employed an ultraviolet spectroscopic method for the assay of plasma from pregnant women and failed to detect any progesterone. Hoffmann and von Lam (1948), using an intra-uterine bio-assay, found a maximum of 10 μg. progesterone/*liter* of human pregnancy blood. It is difficult to reconcile these results with those reported in the present study, although it seems quite possible that some of the blood samples obtained by other investigators, like numerous blood samples assayed for the present study (Figures 1 and 2), contained less than detectable amounts of progesterone.

The plasma progesterone levels presented in Figures 1 and 2 are well within the range of concentrations of the hormone detected during the menstrual cycles of two rhesus monkeys (Forbes, Hooker, and Pfeiffer, 1950a) and four women (Forbes, 1950).

The progesterone levels here reported obviously would not fall on the curve, steadily rising throughout pregnancy, for urinary excretion of pregnanediol (Browne, Henry, and Venning, 1939) and of pregnanediol plus pregnanolone (Venning, 1948). While a full discussion of the relation of pregnanediol excretion to progesterone metabolism is outside the scope of this paper, the reader is reminded that pregnanediol is now regarded by many investigators neither as a metabolite of progesterone alone nor as the only metabolite of this hormone. Secondly, it remains to be shown that the level of a circulating hormone is correlated with the level of a urinary excretion product of that hormone.

Buxton and Atkinson (1948) have reported the basal body temperatures recorded throughout three normal human pregnancies. On the basis of the known ability of administered progesterone to cause an elevation of body temperature, they concluded that the gradual declines observed in the body temperature during these three pregnancies should be interpreted as the reflection of a gradual decrease in circulating progesterone levels.

Neher and Zarrow (1950) followed circulating progesterone levels throughout pregnancy in seven ewes. Their preliminary report indicates an increase to 6 μg. progesterone/cc. serum during early pregnancy, maintenance of this level during approximately the middle trimester of pregnancy, and a further increase by term to 12 μg./cc. In mice, (Forbes, 1948; Hooker and Forbes, unpublished data), on the other hand, the circulating progesterone curve is biphasic, rising to a peak of 7–8 μg./cc. plasma on days 7–9 and again on day 15, dropping precipitatively on day 16, and diminishing thereafter until delivery of the young on day 19. It is evident

that there are conspicuous species differences in the quantities of the hormone circulating during pregnancy.

The possibility that some aspects of the menstrual cycle may not always be entirely obliterated during pregnancy has been suggested by more than one investigator. Hartman (1932), for example, cites several earlier reports of menstruation during pregnancy in a macaque and a chimpanzee, as well as mention of repeated peaks of sexual receptivity in another pregnant chimpanzee. Menstrual bleeding after conception is said to be uncommon in the chimpanzee (Elder and Yerkes, 1932) and macaque (Hartman, 1932). However, one or two increases, roughly at monthly intervals, in the intensity of sex interest (Ball, 1937) and of vaginal desquamation (Hartman, 1932; Ball, 1937) have been reported to be not unusual following conception in macaques. It is the writer's impression that the periodic occurrence of some of the peaks of progesterone concentration (Figures 1 and 2) in the pregnant women and monkeys studied is not due to chance alone.

In the present study, both the low levels of circulating progesterone and the instances when no progesterone was detected are in conspicuous contrast to the much higher concentrations observed in pregnancy in the ewe (Neher and Zarrow, 1950) and the mouse (Forbes, 1948; Hooker and Forbes, unpublished data) and in pseudopregnancy in the rabbit (Hooker and Forbes, 1949a; Forbes, Hooker and Pfeiffer, 1950b). It is well known that, in contrast to the rodents and perhaps some other mammalian groups, the primates do not require the continuing presence of the corpus luteum to insure the successful completion of pregnancy. Pregnancies have gone normally to term without hormonal therapy after bilateral ovariectomy of the macaque as early as the twenty-fifth day after conception (Hartman, 1941; Hartman and Corner, 1947) and of women as early as the forty-first day after the beginning of the last menstrual period (Melinkoff, 1950). Placental production of progesterone has not been measured, at least directly, but in the women and monkeys here considered the placental secretion rate obviously was insufficient to maintain a high circulating level of the hormone. Pearlman (1948) has suggested that the human placental content of progestationally active material may be very small. (The isolated observation on the level of the hormone in the cord blood from H8 supports this idea.) One can scarcely ignore the possibility that not only the corpus luteum but progesterone itself may be unessential for the maintenance of pregnancy in women and monkeys after the first month.

SUMMARY

Bio-assays for free and bound progesterone were performed on peripheral venous blood samples obtained, usually at weekly intervals, throughout most of pregnancy in two women and three monkeys. Concentrations of

bound progesterone were invariably less than 0.6 μg./cc. plasma. Levels of free progesterone were relatively low, not exceeding 2 μg./cc. of human plasma and 3 μg./cc. of monkey plasma. Peaks in concentration of the hormone recurred with some regularity. Peripheral blood levels of progesterone just before and at the time of delivery were more often high than low.

REFERENCES

BALL, J.: *Anat. Rec.* **67:** 507. 1937.

BLOCH, P. W.: *Endocrinology* **20:** 307. 1936.

BROWNE, J. S. L., J. S. HENRY AND E. H. VENNING: *Amer. J. Obstet. and Gyn.* **38:** 927. 1939.

BUXTON, C. L. AND W. B. ATKINSON: *J. Clin. Endocrin.* **8:** 544. 1948.

CLAUBERG, C., H. W. THIEL AND R. ZIECKER: *Arch. f. Gynäk.* **152:** 61. 1933.

ELDER, J. H. AND R. M. YERKES: *Proc. Roy. Soc., Ser. B.* **120:** 409. 1936.

FORBES, T. R.: *Anat. Rec.* **100:** 29. 1948.

FORBES, T. R.: *Amer. J. Obstet. and Gyn.* **60:** 180. 1950.

FORBES, T. R., C. W. HOOKER AND C. A. PFEIFFER: *Proc. Soc. Exper. Biol. and Med.* **73:** 177. 1950a.

FORBES, T. R., C. W. HOOKER AND C. A. PFEIFFER: *Endocrinology* **47:** 83. 1950b.

HARTMAN, C. G.: *Contrib. Embryol. #134, Carn. Inst. Wash. Publ. #433.* **23:** 1. 1932.

HARTMAN, C. G.: *Proc. Soc. Exper. Biol. and Med.* **48:** 221. 1941.

HARTMAN, C. G. AND G. W. CORNER: *Anat. Rec.* **98:** 539. 1947.

HASKINS, A. L., JR.: *J. Clin. Endocrin.* **1:** 65. 1941.

HASKINS, A. L., JR.: *Proc. Soc. Exper. Biol. and Med.* **73:** 439. 1950.

HOFFMANN, F. AND L. VON LAM: *Zentralbl. f. Gynäk.* **65:** 2014. 1941.

HOFFMANN, F. AND L. VON LAM: *Zentralbl. f. Gynäk.* **66:** 292. 1942.

HOFFMANN, F. AND L. VON LAM: *Zentralbl. f. Gynäk.* **70:** 1177. 1948.

HOOKER, C. W. AND T. R. FORBES: *Endocrinology* **41:** 158. 1947.

HOOKER, C. W. AND T. R. FORBES: *Endocrinology* **44:** 61. 1949a.

HOOKER, C. W. AND T. R. FORBES: *Endocrinology* **45:** 71. 1949b.

MELINKOFF, E.: *Amer. J. Obstet. and Gyn.* **60:** 437. 1950.

NEHER, G. M. AND M. X. ZARROW: *Anat. Rec.* **108:** 556. 1950.

PEARLMAN, W. H.: The Hormones. New York, Academic Press. Page 453. 1948.

VENNING, E. H.: *Obstet. and Gyn. Survey.* **3:** 59. 1948.

ACTH-INDUCED CHANGES IN PURINE AND CARBOHYDRATE METABOLISM IN DALMATIAN AND MONGREL DOGS[1,2]

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A DIABETOGENIC effect of ACTH in normal human subjects has been reported (Conn, Louis and Johnston, 1949). An attempt to induce diabetes in mongrel dogs weighing 10–12 kg. was unsuccessful in this laboratory when a daily dose of 26 mg. of ACTH (Armour Standard) was used. Houssay and Anderson (1949) injected dogs, in which 83–87% of the pancreas had been removed, with 50 mg. of ACTH (96% Armour Standard) per kg. per day for four days. Hyperglycemia was observed in only one of the four treated, but glucose tolerance tests were not reported. Campbell *et al.* (1950) showed that ACTH (7.5 mg./kg./day) produced mild hyperglycemia and minimal glycosuria in dogs which were selected for their sensitivity to the diabetogenic activity of anterior pituitary extracts. Overbeek (1950) stated that carbohydrate metabolism was not affected in dogs treated with large doses of ACTH (Organon), but the actual doses employed were not given.

A relationship between ACTH-induced diabetes in man and the associated changes in purine metabolism has been postulated (Conn *et al.*, 1949). Because of the recognized differences in purine metabolism between purebred Dalmatian and mongrel dogs, a comparative study was made of the effect of ACTH upon both purine and carbohydrate metabolism in the two strains.

METHODS

Two Dalmatian and three mongrel female dogs were confined in metabolism cages and fed constant amounts of food each day. All dogs received commercial dog chow (Friskies) except Dalmatian 1, which received raw horse meat and cracker meal in a ratio of 5:1 by weight. These daily rations of food either had maintained the weight of the dogs or had caused a slow gain in weight before ACTH was given. After baseline data

Received for publication April 30, 1951.

[1] Presented in part at the Second Clinical ACTH Conference, Chicago, December, 1950.

[2] This work represents part of a project financed by the Research Grant and Fellowship Division, U. S. Public Health Service.

[3] Research Fellow in Medicine of the American College of Physicians, 1949–1950.

had been gathered during control periods of four to six days, 345 mg. of ACTH[4] (Armour Standard) per day were administered intramuscularly in three divided doses to all dogs for periods of 4–6 days. After cessation of ACTH injections, observations were continued for periods ranging from six to twenty-three days. All dogs were catheterized to obtain accurate 24-hour urine collections. 3 gm. of glucose per kg. of body weight were given in a 20% solution by stomach tube for all glucose tolerance tests performed.

The following chemical determinations were employed: Blood sugar and urinary glucose (Nelson, 1944), blood glutathione (Benedict and Gottschall, 1933), urinary 17-ketosteroids (Robbie and Gibson, 1943), and "11-oxysteroids" (Daughaday, Jaffe and Williams, 1948). Urinary nitrogen determinations were done by the Macro-Kjeldahl method. The determinations for urinary uric acid were performed according to the enzymatic method of Buchanan, Block, and Christman (1945) with the modifications of Bien and Troll (1950) to eliminate interference by urinary glucose. Plasma and urinary allantoin were determined by the method of Christman, Foster, and Esterer (1944), and plasma uric acid by the direct method of Folin (1933). The sum of urinary uric acid nitrogen and allantoin nitrogen values is expressed as "total purine nitrogen."

TABLE 1. RENAL EXCRETION OF ALLANTOIN AND URIC ACID, AND OF "TOTAL PURINE NITROGEN" PER KILOGRAM OF BODY WEIGHT

(Averages for Control Periods)

Dog	Weight kg.	Allantoin mg./kg./day	Uric acid mg./kg./day	Total purine nitrogen* mg./kg./day
Dalmatian				
No. 1	19.1	15.9	33.0	16.5
No. 2	19.8	18.2	36.2	18.8
Average		17.4	34.6	17.7
Mongrel				
No. 5	11.6	56.2	4.1	21.4
No. 3	11.3	54.2	2.4	20.2
No. 7	20.3	47.0	5.2	18.5
Average		52.5	3.9	20.0

* Sum of allantoin nitrogen and uric acid nitrogen.

RESULTS

Table 1 shows the average daily excretions of urinary uric acid, allantoin, and "total purine nitrogen" obtained during the control periods. The excretion of allantoin was three times as great for the mongrel dogs as for the Dalmatians. On the other hand, the uric acid excretion was seven to nine times greater for the Dalmatians than for the mongrel dogs. The excretion of "total purine nitrogen" (sum of allantoin nitrogen and uric acid nitrogen) was approximately the same for all dogs regardless of breed or weight. This confirms the findings of Friedman and Byers (1948) that the sum of urinary uric acid and allantoin nitrogen per day is the same for Dalmatian and non-Dalmatian dogs.

[4] The ACTH was kindly furnished by Dr. E. E. Hays, Director of Biochemical Research, Armour and Company, Chicago.

Fig. 1 shows the effect of ACTH on renal excretion of purines in Dalmatian 1. There was a significant increase in excretion of uric acid during the period of administration of ACTH. The greatest increase in excretion of allantoin occurred during the recovery period. There was an appreciable increase in "total purine nitrogen" excretion during the ACTH period.

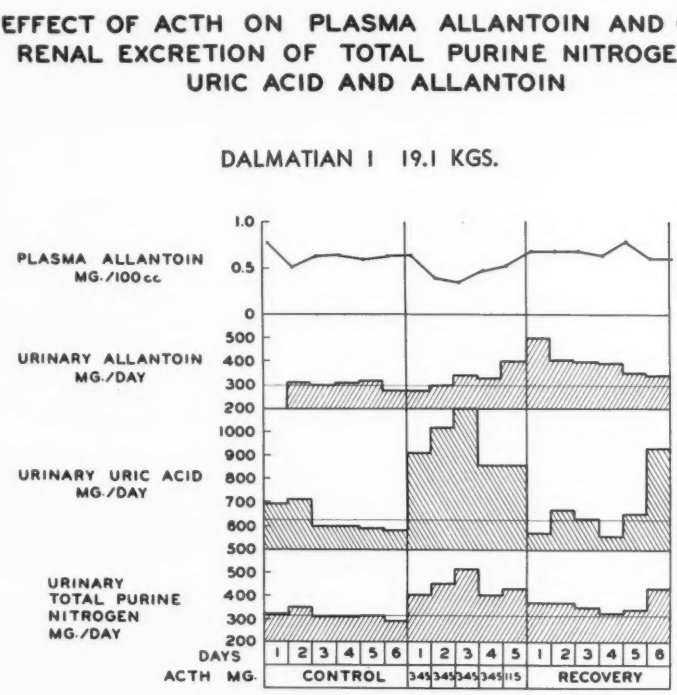

FIG. 1

Fig. 2 shows the data obtained in the second Dalmatian. In this animal ACTH induced a less intense increase in urinary purines. An increase in allantoin excretion did not occur until the recovery period. During the first day of ACTH there was a marked fall in the plasma concentration of uric acid. On the following days there was a rise in concentration of plasma uric acid.

Fig. 3 demonstrates the results obtained with mongrel dog 5. It is observed that the rise in excretion of "total purine nitrogen" was due principally to the rise in allantoin excretion. The plasma concentration of allantoin decreased following the first day of ACTH but then increased to reach maximal concentration in the recovery period. It is to be noted that

renal excretion of allantoin rose at the same time that plasma allantoin was rising and that both values remained elevated during part of the recovery period. The increase in uric acid excretion was not great and was present only during the administration of ACTH.

Fig. 4 shows the data obtained with mongrel dog 3. The plasma con-

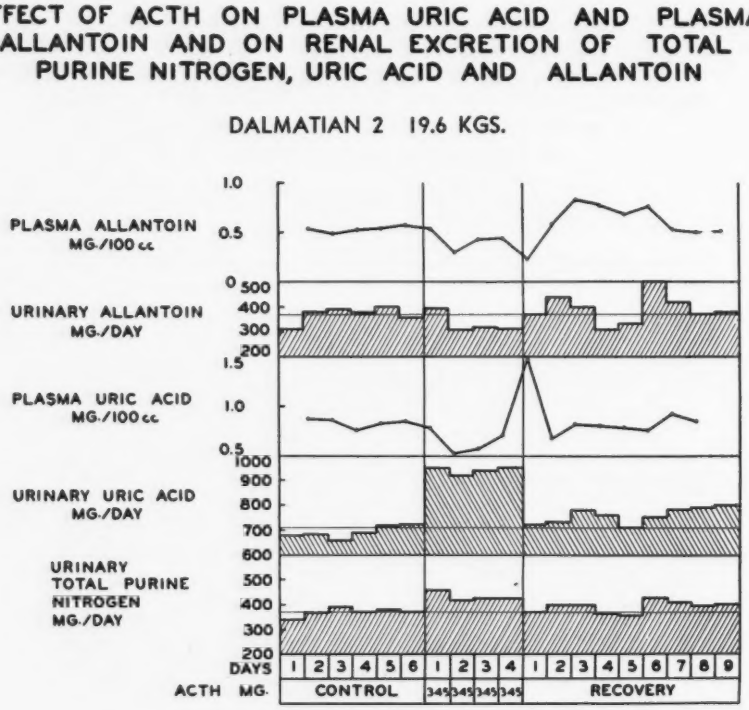

FIG. 2

centration of allantoin did not rise above baseline values until the recovery period and reached a maximum at the end of the fourth recovery day, the day of maximal renal excretion of allantoin. Both values remained elevated through the 15th recovery day. The concentration of uric acid in the plasma remained unchanged during and following ACTH administration. The increase in renal excretion of uric acid was greater than in mongrel 5.

In mongrel 7 the effect of ACTH on plasma concentrations of allantoin and uric acid and on renal excretion of the purines was similar to that in mongrel 3. In mongrel 7 the increases in plasma concentration and renal excretion of allantoin during the recovery period persisted for at least nine days.

Table 2 shows the increase in renal excretion of purines during the ACTH period.[5] The increase in "total purine nitrogen" during the ACTH periods was of the same order for all mongrels and Dalmatian 1. The increase in "total purine nitrogen" excretion in Dalmatian 2 was significantly less.

EFFECT OF ACTH ON PLASMA ALLANTOIN AND ON RENAL EXCRETION OF TOTAL PURINE NITROGEN, URIC ACID AND ALLANTOIN

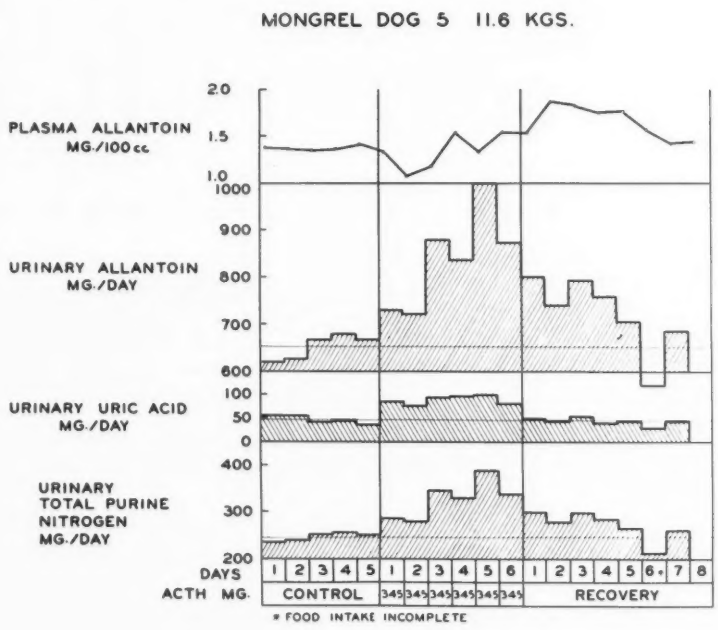

FIG. 3

During the control period the Uricolytic Index[6] averaged 35% for the Dalmatians and 94% for the mongrel dogs. During administration of

[5] It should be emphasized that the values given above (Table 2) for allantoin and therefore for "total purine nitrogen" do not include the increases in allantoin seen during the recovery period. The excretion of allantoin was considerably greater in the recovery period than during the ACTH period in mongrels 3 and 7 and in the two Dalmatians.

[6] Uricolytic Index = $\frac{\text{Urinary Allantoin Nitrogen, mg./day}}{\text{Urinary Allantoin Nitrogen, mg./day} + \text{Urinary Uric Acid Nitrogen, mg./day}}$

EFFECT OF ACTH ON PLASMA ALLANTOIN AND PLASMA URIC ACID AND ON RENAL EXCRETION OF TOTAL PURINE NITROGEN, URIC ACID AND ALLANTOIN

MONGREL DOG 3 11.3 KGS.

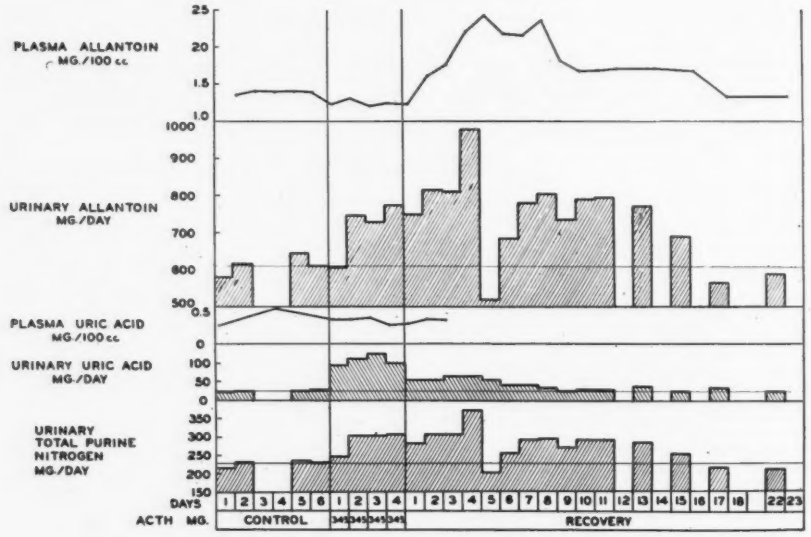

FIG. 4

ACTH there was an average decrease in this index of 8% and 7% for the two Dalmatians and of 3%, 8% and 10% for mongrels 5, 3 and 7 respectively.

Figs. 5–9 illustrate the changes in glucose tolerance induced by administration of ACTH. In both Dalmatians (Figs. 5 and 6) typically

TABLE 2. RISE IN RENAL EXCRETION OF ALLANTOIN AND URIC ACID, AND OF "TOTAL PURINE NITROGEN" DURING ADMINISTRATION OF ACTH (345 MG./DAY). EFFECT OF ACTH ON RENAL EXCRETION OF 17-KETOSTEROIDS, "11-OXYSTEROIDS," AND GLUCOSE TOLERANCE

Dog	ACTH mg./kg.	Rise in allantoin mg./kg./day	Rise in uric acid mg./kg./day	Rise in total purine nitrogen* mg./kg./day	% Rise in 17-keto-steroids	% Rise in "11-oxy-steroids"	Effect on glucose tolerance
Dalmatians							
1	18.1	1.6	16.5	6.3	158%	305%	++++
2	17.6	−1.8	11.7	3.2	154%	200%	+++
Mongrels							
5	30.0	16.0	3.5	7.0	127%	86%	0
3	30.5	9.0	7.0	5.6	81%	31%	+++
7	17.0	6.0	8.2	5.0			+

* Sum of allantoin nitrogen and uric acid nitrogen.

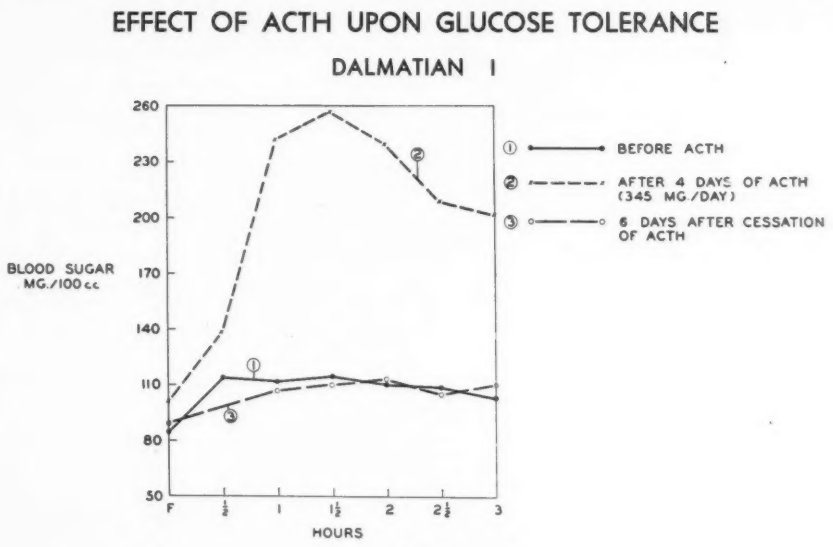

FIG. 5

diabetic glucose tolerance curves were obtained. In mongrel 5 (Fig. 7) there was no change in glucose tolerance. Mongrel 3 (Fig. 8) exhibited a diabetic curve. Mongrel 7 (Fig. 9) showed very mild impairment of glucose tolerance. Glycosuria (maximum of 6.3 grams per 24 hours) occurred only

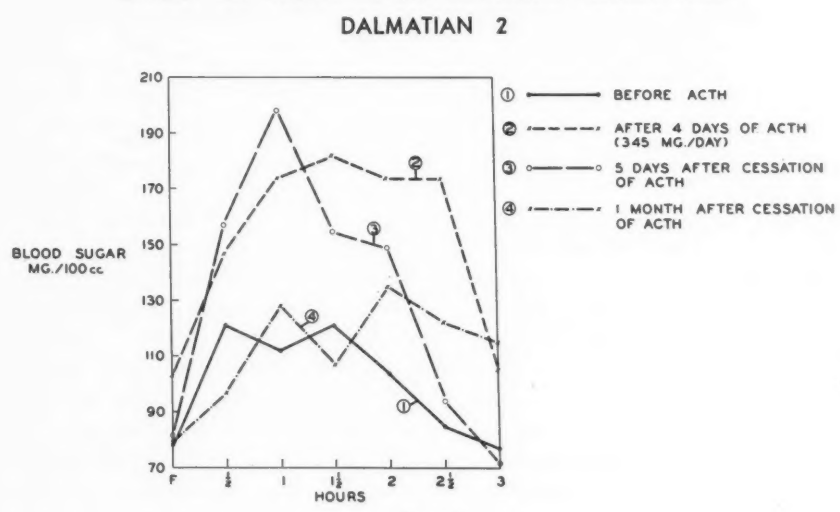

FIG. 6

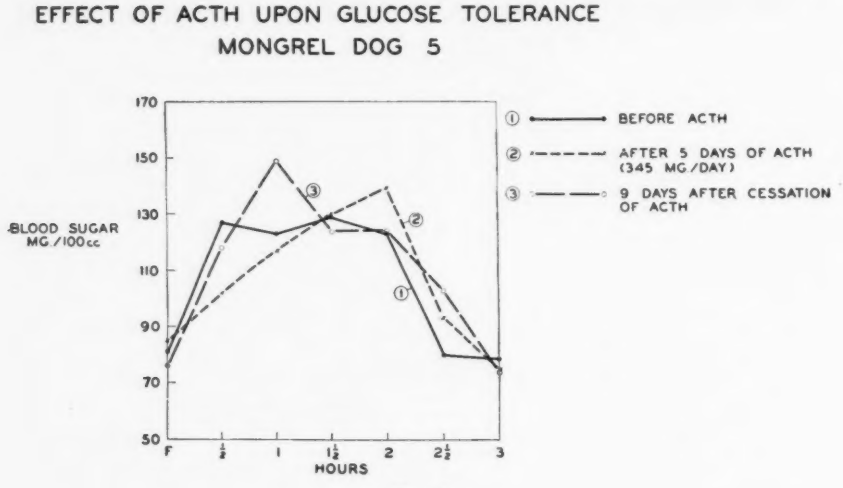

FIG. 7

in Dalmatian 1. This was the only animal in which a significant decrease in blood glutathione was observed (baseline average—29.9 mg. %, average value during ACTH period—22.6 mg. %).

Negative nitrogen balance and increased renal excretion of "11-oxysteroids" and of 17-ketosteroids occurred during ACTH administration

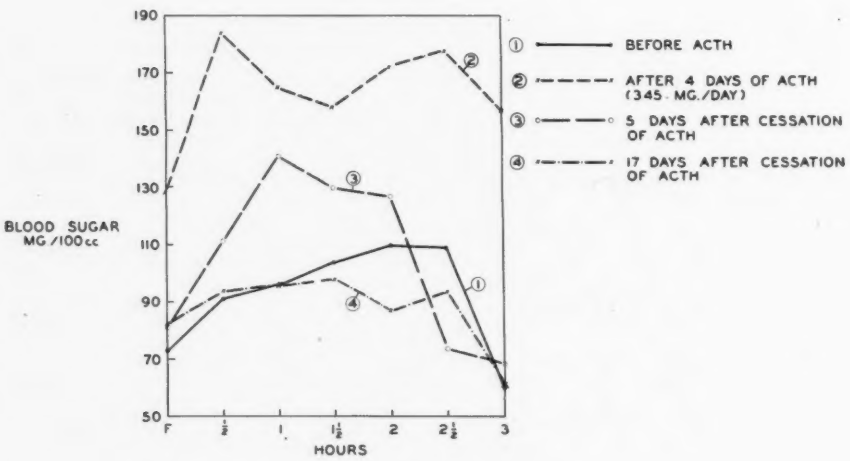

FIG. 8

in both Dalmatians and in the two mongrels in which these determinations were carried out. Fig. 10 shows these changes as they occurred in Dalmatian 1. Table 2 indicates the percentage increases in excretion of "11-oxysteroids" and 17-ketosteroids during administration of ACTH.

DISCUSSION

In all dogs regardless of species there occurred a significant increase in renal excretion of "total purine nitrogen" during ACTH administration. In the mongrels this situation persisted for long periods after cessation of

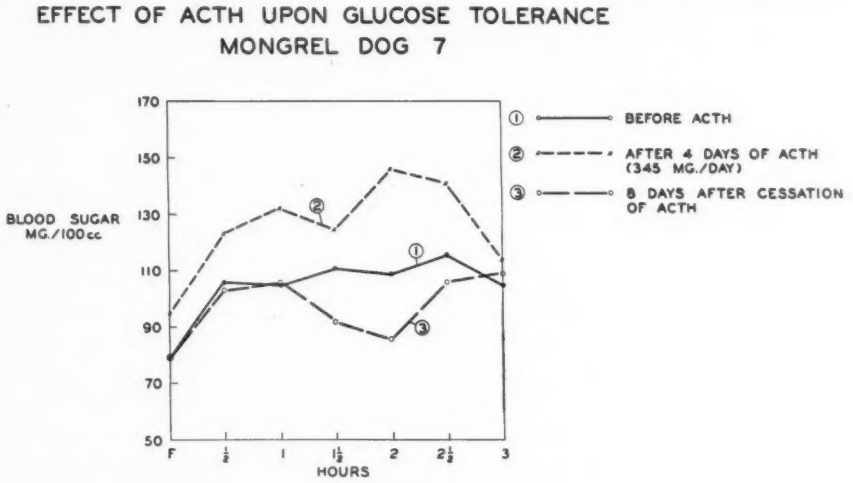

FIG. 9

ACTH. In each breed ACTH caused the greater increase in excretion of that purine which normally constitutes the major excretory product of purine metabolism. Uric acid accounted for the greater increment in the Dalmatians, while it was predominantly allantoin which increased in the mongrels.

In all dogs there was an increase in renal excretion of uric acid during the period of ACTH injections. The data for Dalmatian 2 (Fig. 2) can be interpreted as showing an increase in renal clearance of uric acid on the first day of ACTH in view of the fall of plasma concentration of uric acid on that day. On the following days increased *production* of uric acid is indicated by a rise in concentration of plasma uric acid at a time of elevated renal excretion of uric acid.

In mongrel dog 5 (Fig. 3) there was increased production of allantoin during the period of ACTH administration as evidenced by a simultaneous rise of plasma allantoin levels and renal excretion of allantoin. Increased production of either uric acid or allantoin concomitant with increased ex-

cretion of "total purine nitrogen" can be interpreted as indicating an increase in production of purines during the period of ACTH administration to Dalmatian 1 and mongrel 5. The differences between our results and those of Friedman and Byers (1950) probably can be explained by the difference in the duration of the experiments.

In all mongrel dogs there was evidence for increased production of allantoin during the recovery period in view of the simultaneous elevation of

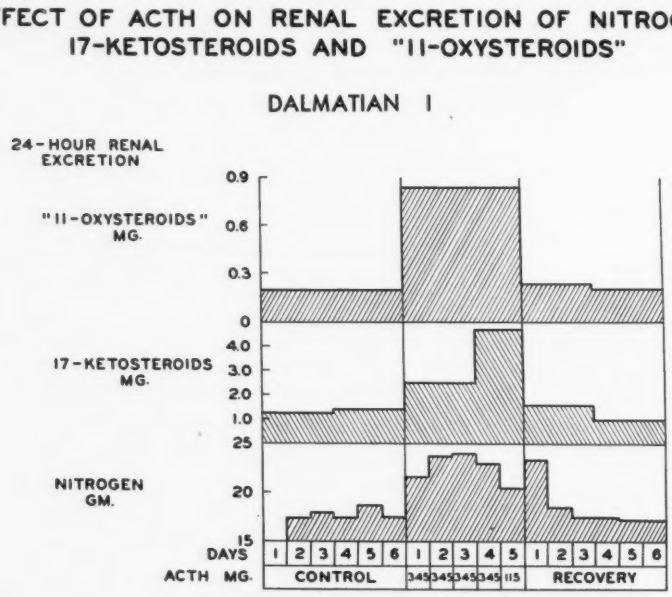

FIG. 10

plasma allantoin levels and an increase in the excretion of allantoin. Since during this period of increased allantoin production the uric acid excretion did not fall below that of the control period, increased purine production must have occurred.

In normal humans an increase in uric acid excretion is observed during administration of ACTH, but this increment disappears promptly upon cessation of ACTH. The prolonged increase in purine production observed in dogs after cessation of ACTH deserves more study. In these animals the increased excretions of "11-oxysteroids," 17-ketosteroids and nitrogen had returned to control levels much earlier.

A decrease in the Uricolytic Index during the ACTH period was observed in all dogs. This could be due to depression of the uricase enzyme system by ACTH inasmuch as the activity of a number of enzyme systems are known to be altered by ACTH (Thorn *et al.*, 1950). On the other hand,

ACTH may alter the manner in which the kidney regulates excretion of either or both of the purine end-products.

Chaikoff, Larson and Read (1935) showed that injection of epinephrine leads to increased renal excretion of allantoin in mongrel dogs and that in Dalmatians it produces a rise in blood levels of uric acid as well as augmented renal excretion of uric acid and allantoin. It had been noted previously that administration of insulin was followed by similar effects on purine metabolism (Larson and Chaikoff, 1935; Chaikoff and Larson, 1935). It was concluded that the action of insulin depended upon the ability of insulin hypoglycemia to effect an increased secretion of epinephrine from the adrenal medulla (Larson and Brewer, 1936). In the light of the results of the present study, the findings of Chaikoff and associates can be explained by the more recently established fact that epinephrine is capable of provoking a pituitary release of ACTH (Long and Fry, 1945).

All dogs received 345 mg. of ACTH (Armour Standard) per day. On the basis of body weight this amounted to between 17.0 and 30.5 mg. of ACTH per kg. (Table 2). Mongrels 3 and 5 received 70% more ACTH per kg. of body weight than the other dogs. Taking this dosage of ACTH into consideration, it appears that the Dalmatians are more sensitive to the diabetogenic effect of ACTH than are mongrel dogs. However, there is no qualitative difference between the two breeds with respect to the effect of ACTH on carbohydrate metabolism.

Table 2 indicates that there is some correlation between the increase in uric acid excretion produced by ACTH and changes in carbohydrate metabolism. The greatest loss in glucose tolerance occurred in Dalmatian 1 which also had the greatest increase in excretion of uric acid per kg. of body weight. Dalmatian 2, which had a rise in purine nitrogen little more than half that of the other dogs, had the second highest increase in excretion of uric acid per kg. of body weight. A moderately severe loss of glucose tolerance was seen in this dog. No change in carbohydrate tolerance was seen in mongrel 5. This animal showed the smallest rise in excretion of uric acid per kg. of all dogs although it demonstrated the greatest rise in excretion of "total purine nitrogen." Mongrels 3 and 7, in which the increases in uric acid excretion were twice that of mongrel 5, showed intermediate degrees of loss of carbohydrate tolerance. As suggested previously (Conn *et al.*, 1949), a mechanistic relationship between ACTH-induced diabetes and ACTH-induced changes in purine metabolism may exist. Partial protection of mongrel dogs against the diabetogenic effect of ACTH may be related to their capacity to convert rapidly uric acid to allantoin. No correlation exists between the percentage rise of "11-oxysteroid" or 17-ketosteroid excretion and decrease of carbohydrate tolerance (Table 2).

These observations suggest that differences in susceptibility of dogs to

the diabetogenic effect of ACTH are related to individual variations in the response of purine metabolism to ACTH.

SUMMARY

Two Dalmatian and three mongrel dogs were given 345 mg. of ACTH per day for periods of four to six days.

Excretion of "total purine nitrogen" was increased about 25% in both strains. Uric acid constituted the major increment in the Dalmatians, while it was predominantly allantoin which increased in the mongrels. Elevated allantoin excretion persisted in the recovery periods for as long as 15 days. Increased production as well as increased clearance accounted for the increment in renal excretion of purines. Both groups exhibited negative nitrogen balance and increased excretion of 17-ketosteroids and "11-oxysteroids."

Typical diabetic glucose tolerance curves were obtained in both Dalmatians and in one mongrel dog. A second mongrel exhibited a very mild decrease in carbohydrate tolerance while a third showed no change. There appears to be a relationship between the diabetic response and the increase of urinary uric acid excretion.

These observations suggest that the differences in susceptibility of dogs to the diabetogenic effects of ACTH are related to individual variations in the response of purine metabolism to ACTH.

REFERENCES

BENEDICT, S. R. AND G. GOTTSCHALL: *J. Biol. Chem.* **99:** 729. 1933.

BIEN, E. J. AND W. TROLL: *Proc. Soc. Exper. Biol. & Med.* **73:** 370. 1950.

BUCHANAN, O. H., W. D. BLOCK AND A. A. CHRISTMAN: *J. Biol. Chem.* **157:** 181. 1945.

CAMPBELL, J., I. W. F. DAVIDSON, W. D. SNAIR AND H. P. LEI: *Endocrinology* **46:** 273. 1950.

CHAIKOFF, I. L. AND P. S. LARSON: *J. Biol. Chem.* **109:** 85. 1935.

CHAIKOFF, I. L., P. S. LARSON AND L. S. READ: *J. Biol. Chem.* **109:** 395. 1935.

CHRISTMAN, A. A., P. W. FOSTER AND M. B. ESTERER: *J. Biol. Chem.* **155:** 161. 1944.

CONN, J. W., L. H. LOUIS AND M. W. JOHNSTON: *J. Lab. & Clin. Med.* **34:** 255. 1949.

DAUGHADAY, W. H., H. JAFFE AND R. H. WILLIAMS: *J. Clin. Endocrinol.* **8:** 166. 1948.

FOLIN, O.: *J. Biol. Chem.* **101:** 111. 1933.

FRIEDMAN, M. AND S. O. BYERS: *J. Biol. Chem.* **175:** 727. 1948.

FRIEDMAN, M. AND S. O. BYERS: *Am. J. Physiol.* **163:** 684. 1950.

HOUSSAY, B. A. AND E. ANDERSON: *Endocrinology* **45:** 627. 1949.

LARSON, P. S. AND G. BREWER: *J. Biol. Chem.* **115:** 279. 1936.

LARSON, P. S. AND I. L. CHAIKOFF: *J. Biol. Chem.* **108:** 457. 1935.

LONG, C. N. H.: *Fed. Proc.*, **6:** 461. 1947.

NELSON, N.: *J. Biol. Chem.* **153:** 375. 1944.

OVERBEEK, G. A.: *J. Clin. Endocrinol.* **10:** 794. 1950.

ROBBIE, W. A. AND R. B. GIBSON: *J. Clin. Endocrinol.* **3:** 200. 1943.

THORN, G. W., *et al.*: *New England J. of Med.* **242:** 783. 1950.

EMBRYONIC MORTALITY IN PREGNANCIES INDUCED IN RABBITS OF DIFFERENT REPRODUCTIVE STAGES[1,2]

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GONADOTROPHINS have been widely utilized to bring about experimental ovulation in a number of species; the fertilizability of such ova in the larger animals appears partially dependent on the stage of the reproductive cycle in which ovulation was induced. Zavadovskii *et al.* (1935) induced heat in cattle treated with gonadotrophins 4–8 days after normal estrus, but failed to obtain pregnancies when these animals were mated or artificially inseminated. Casida *et al.* (1940, 1943) obtained no fertility in cattle treated in the early part of the estrous cycle, but found good fertility in later stages. Similarly, Murphree *et al.* (1944) in the ewe and Tanabe *et al.* (1949) in the sow failed to obtain fertility in animals ovulated during the luteal phase, although fertilization in the follicular phase was satisfactory. Good fertility was also obtained in juvenile, estrual, and anestrual rabbits (Murphree *et al.*, 1947) but none in pseudopregnant females. Boyarsky *et al.* (1947) and Austin (1949) showed that the infertile condition in rabbits developed gradually after the initiation of the pseudopregnancy, but began to disappear with the later regression of the corpora lutea and termination of pseudopregnancy. A similar form of infertility could be produced in estrual rabbits by the administration of progesterone (Boyarsky *et al.*, 1947). The same workers also found some improvement in fertilization in the pseudopregnant treated with estrogen.

Austin (1949) obtained some fertility in pseudopregnant rabbits by deposition of epididymal sperm in the oviducts prior to ovulation. Murphree *et al.* (1951) also found improved fertilization when insemination was directly into the uterus. An appreciable share of the failure of fertilization in the luteal-phase rabbit therefore appears to result from a reduction in the efficacy of sperm transport. Since not all eggs artificially ovulated

Received for publication May 4, 1951.

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Paper No. 454 from the Department of Genetics.

[2] This work has been done under a cooperative agreement between the Wisconsin Agricultural Experiment Station and the Bureau of Dairy Industry, U.S.D.A. The funds contributed by the Bureau of Dairy Industry came as an allotment from the Research and Marketing Act.

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during pseudopregnancy are fertilizable even through use of these special techniques of insemination, the question arises as to whether such ova may not be inherently defective to the extent that they cannot be fertilized. A further question may also be raised as to whether or not many of the fertilizable ova may be slightly defective so that the resulting embryos are incapable of normal development. A study was therefore made of the later development of ova artificially matured and ovulated by gonadotrophin treatment of pseudopregnant rabbits.

Fate of luteal phase ova in situ

Adult rabbits from medium weight breeds were used in this study. Estrual females that had been caged individually at least 30 days and which showed a willingness to accept the male were used. Pseudopregnant females were obtained by mating estrual does to vasectomized males 10 days before re-ovulation. Ovulation was induced in all animals by intravenous injection of 100 I.U. of chorionic gonadotrophin at the time of insemination. Vaginal inseminations were made by exposing the reproductive tract through midventral laparotomy with the animal under light anesthesia and by passage of a 20 gauge hypodermic needle through the anterior vaginal wall until free in the lumen. Uterine insemination was performed in a similar manner, but the needle after puncturing the vaginal wall was passed through each cervical canal until free in the uterine lumen. The semen used consisted of the pooled ejaculates of several males of known fertility collected with an artificial vagina. The semen was then diluted 1:4 with modified Krebs solution (Lardy and Phillips, 1943). Vaginal inseminations were made with 0.5 cc. of diluted semen, uterine inseminations with 0.25 cc. of diluted semen placed in each uterine horn.

In a preliminary trial six pseudopregnant rabbits were uterine-inseminated. Four were autopsied five days post-laparotomy and one each on days six and seven. The oviducts and uteri were removed and flushed with Krebs solution to recover the embryos. In addition, two five-day pregnant animals (resulting from normal mating of estrual does) were autopsied to determine normal blastocyst size. They yielded 13 and five perfectly spherical blastocysts respectively, in which the trophoblast cells were readily visible. The seven-day and one of the five-day pseudopregnant rabbits yielded six and four degenerating blastocysts respectively. In these the inner cell masses were much shrunken, the trophoblasts were transparent and partially collapsed, and the cells were not readily distinguishable. From two of the four remaining pseudopregnant does several fragmenting ova were recovered; the other animals yielded no embryos. Pus which was found in the uteri of some animals may have interfered with recovery.

Observation of the degenerating embryos resulting from fertilized luteal-phase ova in the above trial led to a further test in which the animals were not autopsied until 15 days post-laparotomy. Eight estrual does were vaginal-inseminated, and eight estrual and eight pseudopregnant females uterine-inseminated. The reproductive organs were removed at autopsy, the ovaries examined for the number of new corpora lutea, and measurements made of the embryonic sites. The uteri were opened and the size and condition of the embryos noted. None of the pseudopregnant animals had normal embryos (table 1), while the percentages of corpora lutea accounted for by normal embryos in the estrual groups (vaginal- and uterine-

TABLE 1. EMBRYONIC DEATH IN PREGNANCIES INDUCED IN ESTRUAL AND PSEUDOPREGNANT RABBITS

Reproductive stage	Site of insemination	No. does	No. CL	Percentage of CL accounted for by		
				Normal embryos	Degenerate sites	Missing ova
Estrual	Vagina	8	84	39.3	9.5	51.2
	Uterus	8	84	48.8	9.5	41.7
Pseudopregnant	Uterus	8	109	0.0	6.4	93.6

inseminated) were 39.3 and 48.8 respectively. Pyometra was evident in the uteri of three of the pseudopregnant animals, but this condition was absent in the estrual rabbits. Murphree *et al.* (1951) noted this endometritis in many pseudopregnant females when autopsied 30–45 hours after uterine insemination. Persistence of pus after two weeks would indicate that the endometrium was still inflamed or that resorption of the purulent material was slow.

On the assumption that approximately 60% of the luteal-phase ova in this study were fertilized by the techniques used (Murphree *et al.*, 1951), the lack of viability in such ova might possibly be due to any one of a number of causes. The ova which are artificially ripened and ovulated in an ovary under the influence of progesterone may have inherent defects, not sufficient to prevent fertilization of some of the eggs, but which prevent the later development of the resulting embryos. Another cause for failure may be that the ova are not properly transported to the uterus. A further possibility is that the uterine environment is inadequately prepared for the implantation of the embryo or for its later development and nutrition. The presence of pus in the uteri of these animals might also have some bearing on the viability failure. No effective methods appear available for separating pre- and post-ovulatory factors within the individual female. Accordingly, a further study was undertaken by the transplantation of fertilized ova into other females.

Fate of fertilized ova when transplanted

Twenty pairs of adult rabbits, divided into four groups of five pairs each, were used in the transplantation study. These groups comprised: estrual donor—estrual host, estrual donor—pseudopregnant host, pseudopregnant donor—estrual host, and pseudopregnant donor—pseudopregnant host. Ovulation was induced in all donors by intravenous injection of 100 I.U. of chorionic gonadotrophin (10 days after mating to a vasectomized male in the case of pseudopregnant donors) and were inseminated by laparotomy and injection of Krebs-diluted semen into the upper ends of the uterine horns. The host animals received an intravenous injection

TABLE 2. RESULTS OF RECIPROCAL TRANSPLANTATION OF FERTILIZED OVA FROM ESTRUAL AND PSEUDOPREGNANT RABBITS

Host	Comparison	Donor		
		Estrual	Pseudopregnant	Both
Estrual	No. females pregnant	3[1]	4[1]	7
	Total ova transplanted	18[2]	21[2]	39
	Total embryonic sites	8	14	22
	No. normal embryos	5	14	19
Pseudopregnant	No. females pregnant	2[1]	3[1]	5
	Total ova transplanted	20[2]	22[2]	42
	Total embryonic sites	6	10	16
	No. normal embryos	5	9	14
Both	No. females pregnant	5	7	12
	Total ova transplanted	38	43	81
	Total embryonic sites	14	24	38
	No. normal embryos	10	23	33

[1] Number pregnant out of five host animals.
[2] Total number of ova transplanted into five host animals.

of gonadotrophin to induce ovulation simultaneously with their donor-mates. All donors were autopsied approximately 25 hours after insemination and the oviducts removed and flushed with modified Krebs solution to recover the ova. Five fertilized ova (i.e., equally cleaved, as determined by examination in a small watch glass with a low power binocular microscope), wherever this number was possible, were picked up in a small drop of fluid in a capillary pipette. The hosts were placed under anesthesia and an incision made in the right flank, exposing the right ovary and infundibulum of the oviduct. The capillary tip containing the ova was carefully introduced into the infundibular opening to a depth of approximately one inch and the contents gently expressed, care being taken to minimize the introduction of air; the incision was then closed. Autopsy of the host does was made 14 days later, the reproductive tract removed, the embryonic sites measured and the condition, size, and weight of the embryos noted.

Three of five estrual does receiving follicular-phase ova were pregnant at autopsy (table 2). Of a total of 18 fertilized ova transplanted into the five females, five were developing as normal embryos. By contrast, four of five estrual hosts receiving luteal-phase ova were pregnant, and of a total of 21 ova transplanted, 14 resulted in normal embryos. The difference in embryo survival is in favor of the luteal-phase ova. This would indicate that the fertilized ova obtained from pseudopregnant rabbits by injection of gonadotrophin and uterine insemination are at least equal in viability to ova from estrual does. Whether such luteal-phase ova are actually superior should be tested further.

Only two of five pseudopregnant hosts receiving ova from estrual donors were pregnant; five of 20 transplanted ova survived as normal embryos. Three of five pseudopregnant hosts receiving luteal-phase ova were pregnant, with nine of 22 ova developing normally at time of autopsy. This difference was not significant. But the fact that fertile ova, from either estrual or pseudopregnant donors, can survive in the pseudopregnant host indicates that the uterine environment of such hosts *per se* is not completely at fault in the failure of embryos to survive *in situ*.

Considering the donors only and disregarding the type of host animal, 10 of 38 ova from estrual does survived to autopsy while 23 of 43 ova from pseudopregnant donors resulted in normal embryos. This difference is again in favor of the ova from the luteal-phase animal. When hosts only are considered, 19 of 39 ova transplanted into estrual does yielded viable embryos, while 15 of 42 ova transplanted into pseudopregnant females were viable at autopsy. This difference is not significant.

These results fail to support the hypothesis that artificial maturation of ova in an ovary containing active corpora lutea lowers the viability of the embryos resulting from the fertilization of such ova. Murphree *et al.* (1951) found that approximately 60% of such ova could be fertilized by uterine insemination, whereas about 95% of eggs in estrual females are fertilizable. Further study will be needed to determine whether the failure of fertilization of the remaining 35% of ova in the pseudopregnant is due to inadequate sperm transport and other conditions existing in the oviducts of the luteal-phase animal (as suggested by Austin, 1949), or whether this failure is due to inherent defects in the artificially matured uva. Estimates of fertilization failure and embryonic mortality in rabbits at this laboratory approximate 50% in the normal adult estrual animal. It might be theorized that in any particular crop of eggs ovulated from an ovary some ova contain defects of various orders of intensity. In about 5% of follicular-phase ova these defects are so severe as to preclude fertilization, while in others, defects of lower intensity permit fertilization but cause death of the embryo at various stages in development. In the luteal-phase

animal, on the other hand, treatment may have intensified the latter grade of defects to the point where even fertilization tended to be made impossible. Thus there would be a tendency for defect-free ova only to be fertilized and they would be more likely to develop normally. If this alternative should be the case, then the luteal-phase ova used in this study (since only normally cleaved ova were utilized) may represent a more highly select population in terms of viability than those obtained from estrual does.

A further conclusion may be drawn that the uterine environment of the pseudopregnant rabbit (i.e., one that has been subjected to the influence of progesterone for a considerable period) is not of such a nature as to prevent implantation and subsequent development of the embryo, providing a second ovulation has occurred. The hormonal pattern involved in the re-ovulated pseudopregnant female is not known. The corpora lutea of pseudopregnancy appear somewhat blanched at laparotomy 25 hours after the injection of gonadotrophin, and only remnants of them are found 14 days later. Presumably, the rapid maturation of follicles under the influence of the exogenous gonadotrophin would result in a short period of increased estrogen production with waning progesterone secretion.

Austin (1949) found that fertile ova were not transported through the oviducts of estrual or pseudopregnant rabbits (although cleavage continued normally) except following a recent ovulation. Also, implantation is assumed possible in the normal animal only during a limited time following ovulation. Courrier and Kehl (1930) obtained deciduomata formation following stimulation of the rabbit uterus during days 5–8 but not after day 12. It would appear that the secretion from the second set of corpora lutea in the present study controls the descent and implantation of ova in the pseudopregnant animals, and that these functions are performed in a normal manner judging from the results obtained.

A further point of interest is found in the survival of ova from pseudopregnant does transplanted into pseudopregnant hosts, whereas luteal-phase fertilized ova do not develop *in situ*. The chief difference in these two situations is the lack of uterine insemination in the transplanted host. As noted in the first section of this paper, there was a marked endometritis with pus formation in some pseudopregnant females in which semen was deposited in the uterus. Eight of 10 pseudopregnant donors in this trial also showed pyometra when autopsied 25 hours after the uterine insemination; the condition was not found in the uninseminated pseudopregnant hosts.

The conclusion then follows that the failure of fertilized luteal-phase ova to survive *in situ* is due largely to some condition associated with the pyometra. While reasonable care was taken to use clean inseminating equipment, the semen, due to the method used in its collection, was prob-

ably highly contaminated. The physiology of this condition remains for future investigation. It is significant to note at this point, however, the differential response of the estrual and pseudopregnant uterus to this treatment.

SUMMARY

Adult estrual and pseudopregnant rabbits were ovulated by the administration of gonadotrophin and inseminated either vaginally or intrauterine by laparotomy. At autopsy 15 days later the percentage of corpora lutea accounted for by normal embryos was 39% in vaginal-inseminated estruals, 49% in uterine-inseminated estruals, but 0% in uterine-inseminated pseudopregnant females.

Fertilized ova obtained from estrual and pseudopregnant donor rabbits 25 hours after gonadotrophin-treatment and uterine insemination were transplanted to the oviducts of other artificially ovulated estrual and pseudopregnant host does. At autopsy 14 days later the numbers of normal embryos resulting from the transplanted ova were five of 18 for estrual-to-estrual transplant, 14 of 21 for pseudopregnant-to-estrual, five of 20 for estrual-to-pseudopregnant, and nine of 22 for pseudopregnant-to-pseudopregnant.

It is concluded that the failure of fertilized luteal-phase ova to survive *in situ* is not due to defects in the eggs nor to endocrine inadequacies of the uterine environment, but rather to factors associated with the development of a pyometra in the pseudopregnant uterus into which semen has been placed directly.

REFERENCES

Austin, C. R.: *J. Endocrinol.*, **6**: 63. 1949.

Boyarsky, L. H., H. Baylies, L. E. Casida and R. K. Meyer: *Endocrinology* **41**: 312. 1947.

Casida, L. E., R. K. Meyer, W. H. McShan and W. Wisnicky: *Am. J. Vet. Res.* **4**: 76. 1943.

Casida, L. E., A. Nalbandov, W. H. McShan, R. K. Meyer and W. Wisnicky: *Proc. Am. Soc. Anim. Prod.* p. 302. 1940.

Courrier, R. and R. Kehl: *C. R. Soc. Biol.*, Paris. **104**: 1180. 1930.

Lardy, H. A. and P. H. Phillips: *Am. J. Physiol.* **138**: 741. 1943.

Murphree, R. L., W. G. Black, G. Otto and L. E. Casida: *Endocrinology* (In Press). 1951.

Murphree, R. L., E. J. Warwick, L. E. Casida and W. H. McShan: *J. Anim. Sci.* **3**: 12. 1944.

Murphree, R. L., E. J. Warwick, L. E. Casida and W. H. McShan: *Endocrinology* **41**: 308. 1947.

Tanabe, T. Y., A. C. Warnick, L. E. Casida and R. H. Grummer: *J. Anim. Sci.* **8**: 550. 1949.

Zavadovskii, M. M., I. A. Eskin and G. F. Ovsjannikov: *Trud. Dinam. Razvit.* **9**: 75. 1935. (Cited from *Anim. Breed. Abstr.* **4**: 37).

THE SITE OF THYROTROPHIN AND GONADOTROPHIN PRODUCTION IN THE RAT PITUITARY STUDIED BY McMANUS-HOTCHKISS STAINING FOR GLYCOPROTEIN

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ADAMS (1946) has summarized the evidence for the source of thyrotrophin in the pituitary. At that time the evidence available was divided between the two types of chromophil cells favouring the acidophils rather than the "basophiles," while more recent results tend to support the view that the "basophil" cells are the source of thyrotrophic hormone secretion. The question, however, is not regarded as settled. In studies at this laboratory (Griesbach, 1941; Griesbach and Purves, 1945) we showed a correlation between increases in the number and size of "Basophil" cells and an increasing thyrotrophin secretion. These changes were induced by small degrees of thyroxine deficiency which produced no change in the acidophil cells. It was shown (Purves and Griesbach, 1946a) that the acidophil cell degranulation which follows total thyroidectomy is due to a failure of pituitary function in the complete absence of thyroxine. It, therefore, seemed to us that the "basophil" was the source of thyrotrophin. Salter (1950) arrived at similar conclusions in respect to the "basophil" cells.

We are well aware of the fact that the term "basophil" is incorrect when applied to the rat's and similar animal's pituitaries. We believe that the term cyanophil is more appropriate as it indicates the property of these cells to stain blue with aniline blue in the Mallory or Azan techniques. However, the term "basophil" is so deeply entrenched in the literature that it is almost impossible to discuss the recent work without introducing this term. In this paper the terms cyanophil or "basophil," with quote marks, will be used to denote the cells which stain with aniline blue and the term basophil, without quote marks, will be reserved to denote true basophilia as revealed by an affinity for basic dyes.

The description of a new technique (McManus, 1946) of staining with periodic acid followed by Schiff's reagent has permitted the demonstration of glycoprotein in histological sections. The technique was elaborated by Hotchkiss (1948) and has been applied with conspicuous success by Catchpole (1949) to the study of the hormone content of the pituitary.

Received for publication May 4, 1951.

Using frozen dried material, Catchpole was able to demonstrate in the cyanophil cells an accumulation of granules which gave a positive periodic acid-Schiff reaction (PAS) for glycoprotein and to show that these granules had solubilities corresponding to those of purified FSH and LH as isolated from pituitary glands. He, moreover, showed a correlation between the content of these granules in female rats with the stage of the oestrus cycle. In the pituitary glands of the thyroidectomized male rats he recorded an increase in the amount of demonstrable glycoprotein in cyanophils, but the solubility of these granules was unlike that of thyrotrophin.

From the result of previous assays of rat pituitaries for thyrotrophin (Griesbach and Purves, 1943; Purves and Griesbach, 1946b) we found the highest concentration of thyrotrophin in the normal rat pituitary. In thyroxine deficiency the concentration was lower and since the "basophils" were then much larger and more numerous, the thyrotrophin, if it were in them, would be at a low concentration in their cytoplasm. On the other hand, when thyroxine was administered in large doses, the thyrotrophin content fell nearly to zero (Purves and Griesbach, 1946b). We, therefore, sought to demonstrate a glycoprotein corresponding to thyrotrophin which would be maximal in normal rat pituitaries.

Pearse (1949) has applied the McManus-Hotchkiss technique to human pituitaries after fixation in routine fixatives such as Helly, Zenker, half-saturated mercuric chloride in formol-saline or formol alone and has shown periodic acid Schiff positive granules in the basophil cells. We have, therefore, investigated the staining of the rat pituitary after fixation in 10% formalin in saturated mercuric chloride which is our routine pituitary fixative.

METHOD

After fixation in formol sublimate (10% formalin, 90% saturated $HgCl_2$), paraffin sections were prepared at 2.5 micra and 5 micra thicknesses. The 5 micra sections were used for the periodic acid-Schiff staining. The 2.5 micra sections were stained by a modification of the Mallory stain (Martins, 1933). Since comparative tests show that the use of the alcoholic solutions introduced by Hotchkiss with the purpose of retaining glycogen during periodic acid-Schiff staining were unnecessary for the staining of fixed pituitary material, aqueous solutions were used as originally used by McManus. Moreover, the intermediate reducing rinse of Hotchkiss was found to be unnecessary when staining sections since the Schiff reagent contains a large excess of sulphurous acid which reduces any normal amount of periodates remaining in the section.

The solutions required are:

A. 0.5% periodic acid (HIO_4).

B. Schiff's solution prepared according to Coleman. (Glick, 1949, page 67.)

C. Haematoxylin (Harris' H. Solution).

Procedure: It was found most economical to stain the sections on the slides. After deparaffinization and passage through iodine solution and thiosulphate to remove mercury precipitate, the slides were washed for half an hour and then treated as follows:

(1) 10 minutes with periodic acid solution A on the slide at room temperature.
(2) Short rinse.
(3) Schiff's solution on the slide for 30 minutes.
(4) Wash in running water 30 minutes.
(5) Differentiate if necessary in 70% alcohol followed by short rinse.
(6) Stain in Harris' haematoxylin for 30 seconds. Blue in running water for 10 minutes.
(7) Dehydrate with absolute alcohol, clear and mount in balsam.

For localization of the different cell types, numerous pituitaries were sectioned at 5μ in series in three planes, horizontal, coronal and sagittal. Of the horizontal section every 10th., of the coronal every 40th. section was photographed and enlarged.

For the purpose of producing variation in the thyrotrophic hormone content of the pituitary the thyroxine level was varied either by thyroidectomizing rats or by administering 0.01% methylthiouracil in the drinking water in order to produce thyroxine deficiency. Thyroxine excess was produced by injecting 5 to 10 μg. of DL-thyroxine per day for 21 days, or a peptic digest of thyroideum sicc. in amounts supplying 25 to 50 μg. of thyroid iodine per day. To produce variation in the gonadotropin hormone content, rats were castrated or were treated with oestradiol benzoate in peanut oil in amounts of 1, 2 and 5 μg. per day for 3 to 6 weeks.

The rats used were a local albino strain and were 120–150 grams in weight at the time of use.

RESULTS

(1) *Identity of cyanophilic granules with PAS positive granules.* In the normal rat pituitaries PAS stained certain cells in the pars anterior as described by Catchpole (1948). In addition to these cells the following structures are stained:—the colloid of the cleft between the pars intermedia and the pars anterior, the cells of the pars intermedia and the reticulum throughout the gland. The plasma within the blood vessels also takes a slight pink colouration. In this paper only the staining of the cells of the pars anterior is considered.

For confirmation of the observation of Catchpole that the cells containing glycoprotein are cyanophils, use was made of counter staining the PAS section with phosphotungstic acid and fast green. The combination of the green staining on the previously purplish red PAS positive cells produced a blue colour. In such sections it was possible to search for glycoprotein reacting material which might not be stainable with the phosphotungstic fast green. Cells containing such granules would appear red as in contrast to the blue in those responding to both stains. In normal pituitaries it was rare to find any cells PAS positive and not stained by the fast green. In pathological rat pituitaries, however, numbers of such cells were seen. These were considered to be mast cells or histiocytes. The conclusion was reached that in the rat there were no chromophobes which were PAS positive.

Counter-staining with fast green stains the acidophils as well as the

cyanophiles. To check whether the acidophils ever contain PAS positive material, use was made of counter staining by phosphotungstic acid-Orange G as used by Pearse (1949). After differentiating in running water, a preparation is obtained by which the acidophils and red blood cells are stained orange. In such sections no PAS positive material was ever seen in acidophils.

As a further check of the identity of the PAS positive cells with the cyanophils, sections stained by Martin's modification of the Mallory stain were examined under the microscope and selected fields were photographed. The slides were then restained by the PAS procedure and the same fields rephotographed. The appearance produced by the PAS corresponds with great accuracy to the appearance produced by the aniline blue staining of the Mallory method. This correspondence was so precise to suggest that the same granules are stained by the two methods. This correspondence of the blue staining in the Mallory method to the red staining by PAS is true also of the reticulum and the cells of the pars intermedia. We conclude, therefore, that there is an exact correspondence between the cyanophils and the PAS positive cells because the two methods are, in fact, specific for the same cytoplasmic materials.

(2) *Functional response of PAS positive cells to sex hormone disturbances.* Early in the investigation of the responses of the PAS cells, it became apparent that these cells could be divided into two groups with quite distinct specific responses. The first group, which on the evidence to be presented later was termed "gonadotrophs," responded to the injection of oestrogen by complete loss of PAS granules. These cells are identifiable in the normal pituitary partly by their location and partly from difference in shape and staining from the second group. They are round or oval and give an intense reaction with PAS which makes them conspicuous under low power during the staining process. Many of them show the negative image of the Golgi apparatus and, in older animals, they quite frequently show the castration type of the signet ring cell. The granules are coarse and, when a vacuole is present, the granules surround the vacuole. A most striking feature is the frequent attachment of these cells to the big portal vessels. Where they occur at some distance from the larger vessels they stick to the smaller vessels like fruit to a branch (Figs. 2 and 3).

We have tried to localize these cells in the pituitary as it appeared that they can be found in greater numbers and with some regularity in certain areas. Serial sections were cut on entire pituitaries in the three main planes and after staining with PAS the distribution of the "gonadotrophs" cells was mapped out on low power photographs or on tracings prepared from sections at regular intervals throughout the glands. The horizontal sections nearest the inferior surface of the pituitary (next to the bone)

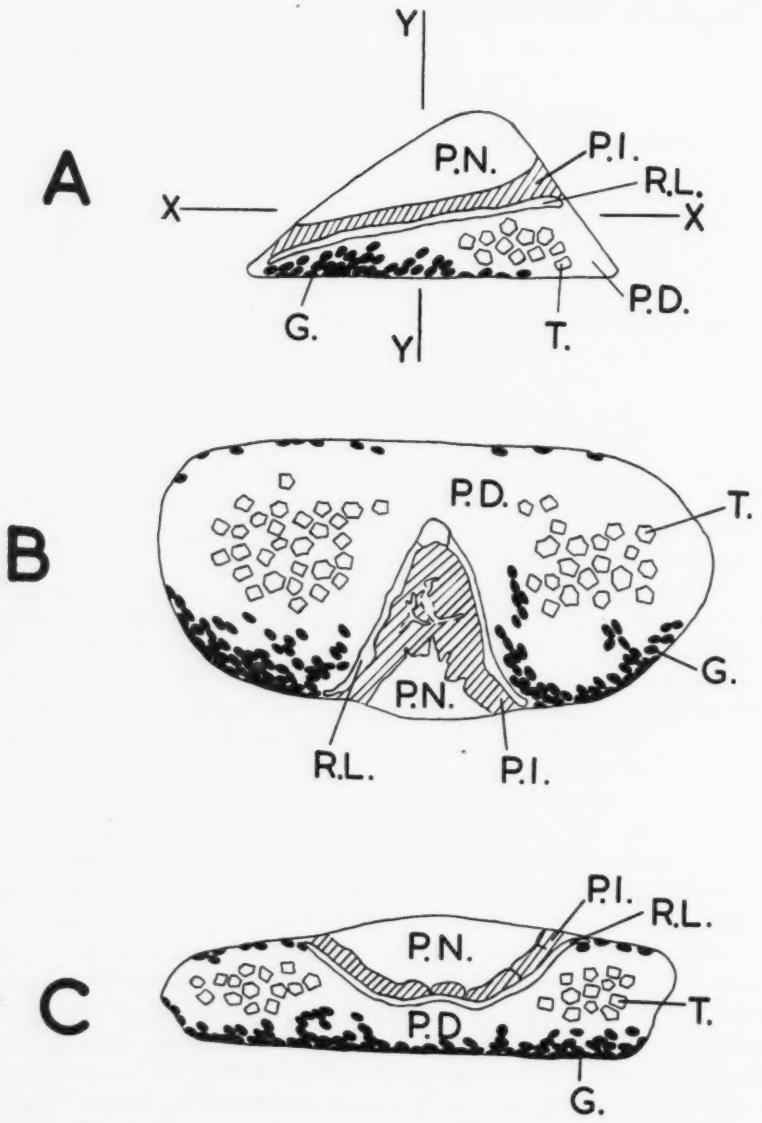


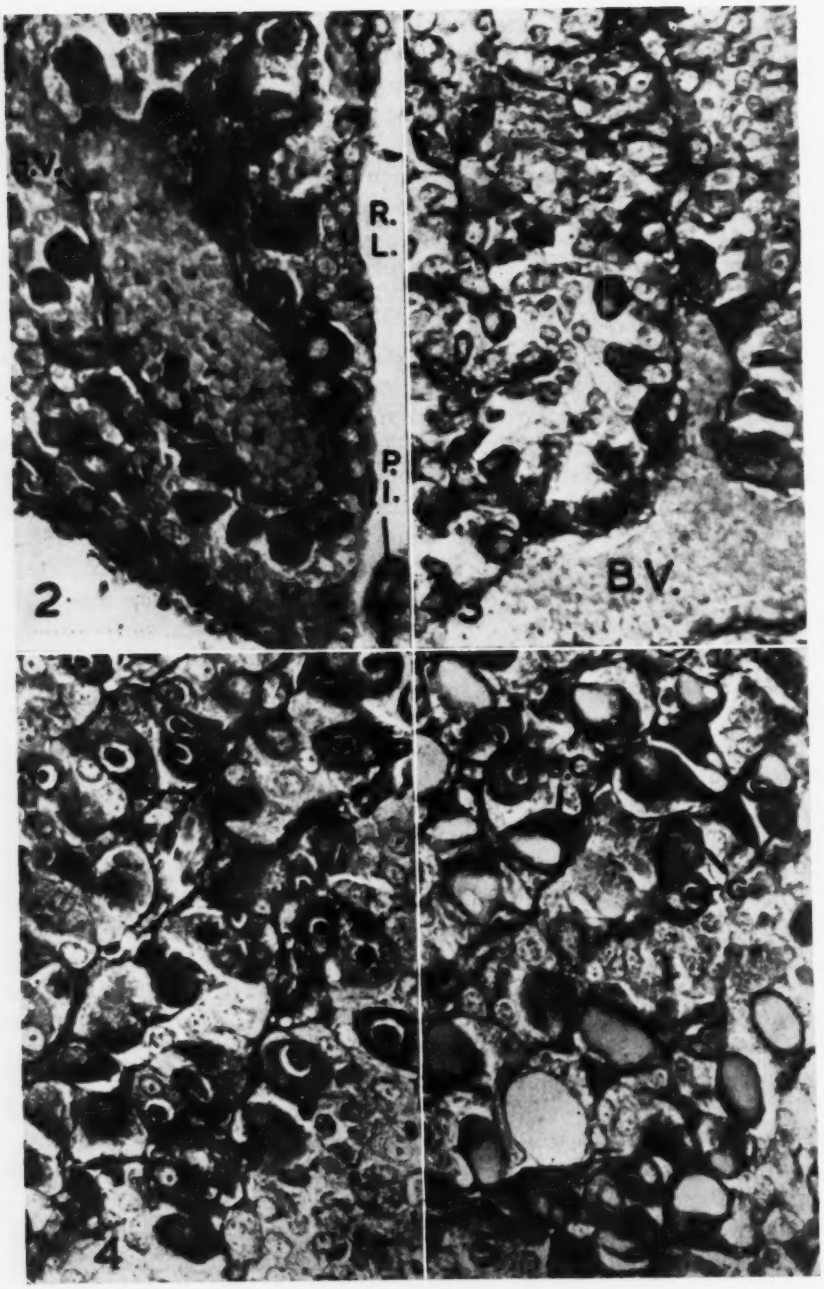
FIG. 1. The distribution of the two types of glycoprotein containing cells in the anterior pituitary of the male rat. A is a sagittal section of the rat pituitary. B is a horizontal section in the plane marked X-X in A. C is a coronal section in the plane marked Y-Y in A. P.N. = Pars nervosa. P.I. = Pars intermedia. R.L. = Residual lumen or cleft. P.D. = Pars distalis or anterior lobe. G. = Gonadotrophs. T. = Thyrotrophs.

show by far the greatest number of the "gonadotrophs." They are crowded around and between the larger portal vessels forming clusters of cells. They are also found in two bands running along the superior surface of the anterior pituitary, one on each side of the posterior lobe, adjacent to the lateral edge of the pars intermedia. In sections in the coronal plane it can be seen that these bands extend somewhat medially beneath the lateral edge of the pars intermedia. These bands thin out posteriorly in similar manner to the sheet of "gonadotrophs" on the inferior surface and do not reach the posterior border which is substantially free from such cells. The greatest density of the "gonadotrophs" is found at the anterior border of the pituitary where the lower sheet of "gonadotrophs" and the two upper bands have a common origin. This distribution is illustrated in Fig. 1, where three mutually perpendicular sections are shown with a diagrammatic representation of the distribution of the PAS positive cells.

The well known castration cells seen in both sexes after gonadectomy are, apparently, derived from the "gonadotrophs." One to two days after castration a conspicuous increase in size and number of the latter cells is observed, starting from the "sex zones" described above and spreading during the next days over the whole pituitary. There are many mitotic figures and the Golgi bodies are enlarged (Fig. 4). The PAS reaction in the granules of the newly formed cells is not so intense but clearly positive. When after several weeks the signet ring cells appear, the hyaline vacuole shows a slight tinge of pink in contrast to the now intensely red stained granules which surround it completely (Fig. 5).

When the normal male rat is injected for 10–14 days with daily doses of estrogen (1–5 μg. of estradiol benzoate in soya bean oil per day), the reaction in the "gonadotrophs" is the opposite one to castration. The "sex zone" seems to be wiped out, but high power inspection reveals that the cells are either still present showing a pink colour, but no granules; or, with the higher dose (5 μg.), are showing signs of degeneration (vacuolation of cytoplasm and regressive changes in the nucleus). The absence of PAS staining cells in the normal "sex zone" after oestrogen is illustrated in Figs. 6 and 7. The effect of oestrogens on the "sex zone" of the female rat has not been investigated. The zone is small in the normal female and PAS positive granules have only been seen during diestrus. We also have no experience with pregnancy pituitaries.

(3) *Response to thyroid disturbances.* The second functional group of PAS positive cells is considered, on the evidence which follows, to be thyrotrophic in function and we have, accordingly, called them "thyrotrophs." These cells are found in the central region throughout the whole anterior lobe. They do not show the predilection for the larger blood vessels that is shown by the "gonadotrophs." Their form is irregular poly-


B.V.
R.
L.
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4

gonal (see Fig. 8). The size is larger than that of the "gonadotrophs." Their granules are fine and do not always give a strong PAS reaction. The negative image of the Golgi is not obvious and quite often not visible.

Following thyroidectomy there occurs the formation of large numbers of "thyroidectomy cells" which, apparently, originate from the "thyrotrophs." Many of them show mitotic figures and they occupy the central region of the anterior pituitary where they replace the normal "thyrotrophs." During the early stages up to 3–4 weeks, the reaction with PAS is only slight (Fig. 9). A pink colouring, due to a fine granulation, can be seen. The hyaline vacuoles, appearing at the second and third week, stain darker than the cytoplasm and on the whole darker than the castration cell vacuoles. At this period, a few cells only can be found which contain intensely stained fine granules within and round the Golgi ring. From 4–6 weeks after thyroidectomy, rarely earlier, the whole picture changes as far as the PAS reaction is concerned. A large number of thyroidectomy cells contain dark red stained particles in their cytoplasm (Fig. 10). These are of varied shape and size. The largest resemble droplets as seen in preparations stained for fat, while others are much smaller and are just visible under high magnification, others are rod or crescent shaped. When part of the cytoplasm is replaced by vacuoles, these particles are always found outside the vacuoles pushed towards the cell border or localized in the fine strand of cytoplasm which surrounds or separates individual vacuoles. As could be expected, the changes described are identical in animals treated with goitrogens. They are still present after long term thyroxine deficiency (one year after thyroidectomy or two years of goitro-

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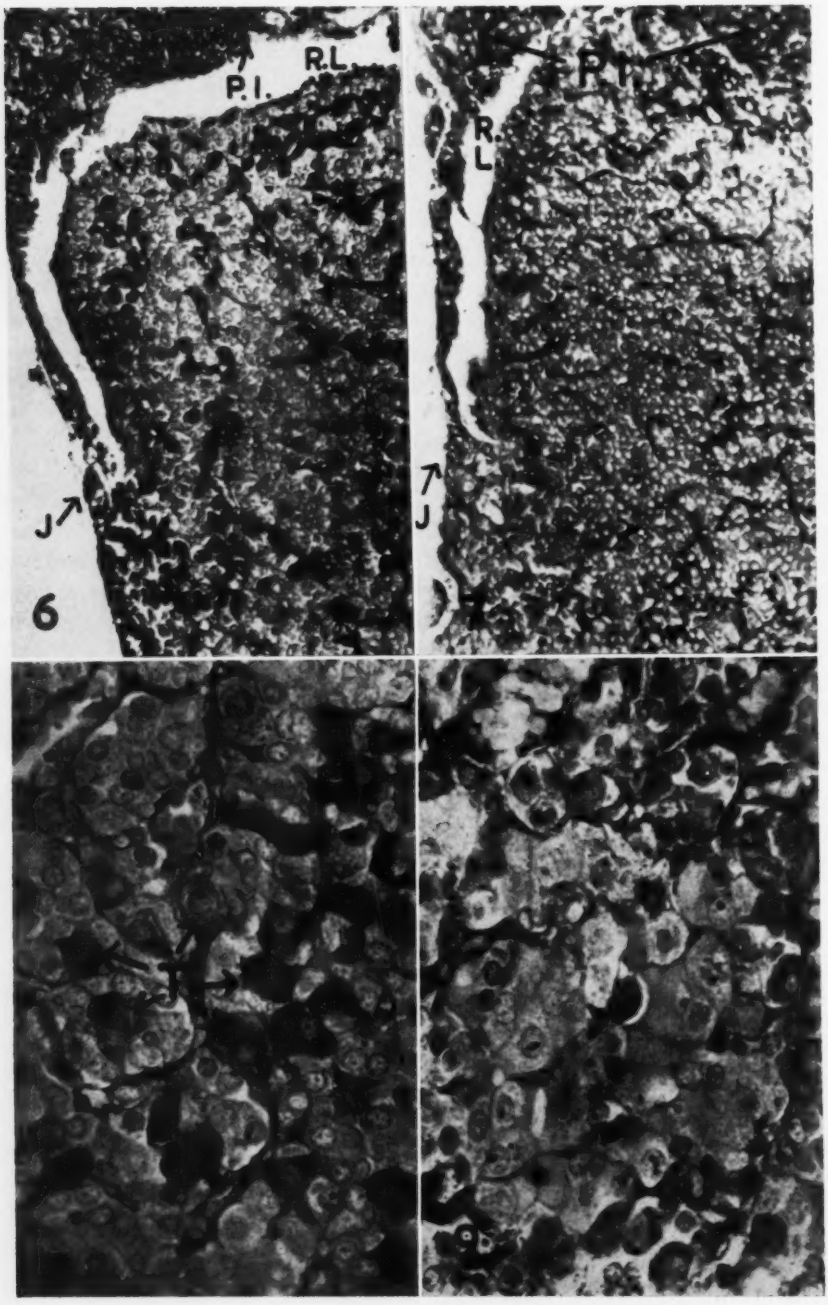
FIG. 2. Section of male rat pituitary showing region at the anterior border adjacent to residual lumen (R.L.) and pars intermedia (P.I.). Gonadotrophs surrounding a blood vessel (B.V.), are round or oval and contain dark staining granules. The reticulum and traces of plasma between the red blood cells also stain with PAS. The nuclei of all cells are faintly stained with Haematoxylin. (PAS and Haematoxylin, ×490.)

FIG. 3. Similar section to Fig. 2, showing gonadotrophs in contact with blood vessel (B.V.) and its branches. (PAS and Haematoxylin, ×490.)

FIG. 4. Pituitary of female rat showing early castration changes (26 days after spaying). The gonadotrophs which have appeared since spaying are larger than the normal gonadotrophs, but have retained the characteristic oval shape. The cytoplasm is stained a pink colour and there is a variable amount of glycoprotein present in discrete granules. Negative images of the Golgi apparatus are very prominent. The glycoprotein reaction is strongest in the Golgi body within the Golgi apparatus. (PAS and Haematoxylin, ×490.)

FIG. 5. Pituitary of male rat showing late castration changes (15 months after operation). Some stimulated non-vacuolated gonadotrophs are present (G) showing the prominent Golgi body. The majority of the gonadotrophs have been converted into signet ring cells (S.C.). The vacuoles are single containing a hyaline substance stained faint pink and they are surrounded by a line of cytoplasm with intensely stained granules. (PAS and Haematoxylin, ×490.)


P.I.
R.L.
J
6
P.I.
R.L.
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gen treatment). They have also been found in a "basophil" adenoma of the pituitary of a rat which had been treated with methylthiouracil for two years. These intensely stained droplets appearing in the thyroidectomy cells are not revealed by the Mallory stain and, therefore, differ from the PAS positive material of the normal pituitary. These granules are evidently those seen in thyroidectomized animals by Catchpole (loc. cit.) who found that their solubility did not correspond with that of the thyrotrophic hormone.

The effect on the pituitary cyanophils of introducing additional thyroxine by injection is apparently complicated. Two phenomena have been consistently observed after giving daily injections of 5–10 μg. of DL-thyroxine or a digest of thyroid substance in dosage containing 25–50 μg. of organically bound iodine. There is a regressive change in the central group of cyanophil cells with loss of PAS positive granules (Fig. 11). This makes it difficult to find them. With Mallory staining they can be seen as chromophobe shadows of such cells which normally show blue staining granules. The nuclei are pycnotic. This picture is obtained with the lower dose of 5–10 μg. thyroxine per day for 19 days. On supplying the higher dose, as thyroid digest, an increase of cyanophils can be seen as described by Severinghaus, Smelser and Clark (1934). The PAS reaction shows a wide spread of "gonadotrophs" trespassing on to ground where they are not usually found. They show increased size, large Golgi bodies and, not rarely, signet ring cells of the castration type (Fig. 12). The surrounding tissue appears quite pale with an occasional pink tinge in one of the regressing "thyrotrophs."

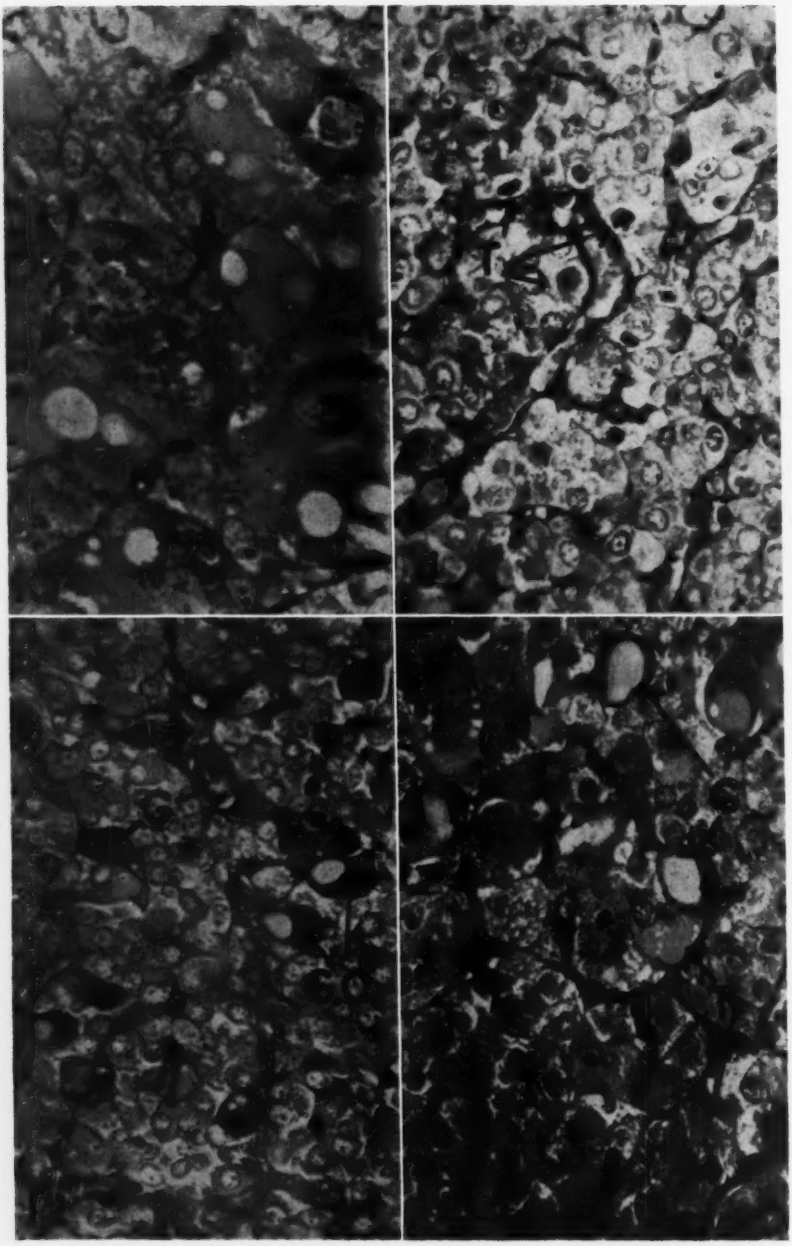
Pituitaries of rats, male and female, gonadectomized and thyroidectomized at the same time and killed after varying intervals up to 8 weeks, when stained with PAS, show the castration and thyroidectomy type of deficiency cell in the same field. They can easily be distinguished (Fig. 13).

FIG. 6. Low power section of male rat pituitary at the anterior border, adjacent to the residual lumen (R.L.) and pars intermedia (P.I.). The gonadotrophs are shown darkly stained and there is a dense collection of them at the junction of the pars intermedia with the pars distalis (J.). (PAS and Haematoxylin, ×170.)

FIG. 7. Low power section of male rat pituitary showing the same region as Fig. 6. The rat had been treated with oestradiol benzoate, 1 μg. for 21 days. The gonadotrophs have disappeared from their normal site. Notice their absence at junction (J) as compared with Fig. 6. (PAS and Haematoxylin, ×170.)

FIG. 8. Section of central region of pars distalis of normal rat. The glycoprotein containing cells (T.) are the thyrotrophs. They are polygonal in outline. The Golgi appartus is inconspicuous. The cells contain varying amounts of glycoprotein granules. There is no close association with blood vessels. (PAS and Haematoxylin, ×490.)

FIG. 9. Section of central region of anterior pituitary of male rat, 21 days after thyroidectomy. The thyroidectomy cells are polygonal in outline, larger than the normal thyrotrophs, with only a faint glycoprotein reaction. Some cells with multiple vacuoles containing hyaline substance. Contrast with Fig. 4. (PAS and Haematoxylin, ×490.)



DISCUSSION

The significance of the glycoprotein staining in the pituitary by the PAS method may be discussed under a number of headings: (1) differentiation of two specific types of glycoprotein containing cells: (2) relationship of the glycoprotein, demonstrated histologically, to the hormone content of the gland: (3) relationship of glycoprotein granules to cyanophils and basophils: (4) localization of site of production of individual hormones.

(1) *Differentiation of two specific types of glycoprotein containing cells.* We have been able to observe two types of cells containing glycoprotein granules in the anterior pituitary of the rat. These types differ in regard to form, affinity to the blood vessels, regional distribution, size and stainability of granules and reaction to changes in the hormonal environment. Their properties are tabulated in Table 1. The most important features in the differentiation of the two types are the functional responses to variation in the hormone supply. It will be seen that when only minor degrees of excess are considered the centrally situated group of cells responds only to the thyroxine fluctuations and is unaffected by changes in the amount of gonadal hormone. On the other hand, the cells situated in the zones which we have indicated in Fig. 1 as containing gonadotrophic cells respond only to fluctuations in the gonadal hormones and are unaffected by variations in the amount of thyroxine. This specificity of response holds true only for moderate or physiological levels of hormone dosage. Gross excess of oestrogen depresses both types of glycoprotein cells. There is similarly an effect of gross and prolonged thyroxine excess which is not at present understood, but which results in an increase in the amount of glycoprotein

FIG. 10. Section of same region of male rat 66 days after thyroidectomy. There is extensive accumulation of hyaline substance which itself contains clear vacuoles. Some of the cells contain coarse intensely staining material of unknown nature which differs from the glycoprotein granules of the normal thyrotrophs. (PAS and Haematoxylin, ×980.)

FIG. 11. Central region of male rat pituitary after 6 weeks of thyroxine administration. (For comparison with Fig. 8.) The glycoprotein reaction of the thyrotrophs has been abolished, but polygonal cells without PAS reaction are still distinguishable. (T.) (PAS, Haematoxylin and Fast Green, ×490.)

FIG. 12. Another part of central region of same pituitary as Fig. 11 showing the appearance in this region of gonadotrophs after thyroxine administration. The gonadotrophs are either stimulated with intense PAS reaction and prominent Golgi bodies (G) or form vacuolated signet ring cells (S.G.). In this and the preceding figure the acidophils can be distinguished as small cells with distinct grey cytoplasm due to counter staining with fast green. (PAS, Haematoxylin and Fast Green, ×490.)

FIG. 13. Central region of anterior pituitary from male rat 8 weeks after castration plus thyroidectomy. Both castration cells (G) and thyroidectomy cells (T) can be distinguished. The characteristic features of both can be observed. (PAS and Haematoxylin, ×490.)

in the gonadotrophic cells. The changes taking place in the gonadotrophic cells in severe thyroxine excess resemble those produced by gonadal deficiency though they are less extensive and are possible due to an impairment of gonad function (Severinghaus, 1937). When one takes consideration only of the immediate response to fluctuations in the hormones not too far removed from physiological levels, there is, however, complete specificity of response.

TABLE 1. CHARACTERISTICS OF TWO TYPES OF GLYCOPROTEIN CONTAINING CELLS IN THE RAT PITUITARY

	Gonadotrophic cells	Thyrotrophic cells
1. Distribution:		
(a) Region	Peripheral, mainly along the under surface and also along the upper surface adjacent to the pars intermedia	Central.
(b) Affinity to blood vessels.	Surround large portal vessels.	None or only sinusoids.
2. Appearance:		
(a) Form	Round or oval	Polygonal
(b) Granules	Coarse, dark red staining.	Fine, pink to red.
3. Reaction to Hormones:		
(a) Thyroxine deficiency. Short term.	No change	Reduction of glycoprotein content. Increase in size and number of cells.
(b) Thyroxine deficiency. Long term.	May be increased in numbers	After one month appearance of abnormal glycoprotein droplets.
(c) Minor thyroxine excess.	No change	Total loss of glycoprotein. Cell regression.
(d) Gross thyroxine excess.	Increased numbers, signet ring cell formation.	Total loss of glycoprotein. Cell regression.
(e) Gonadal deficiency. Early	Increase in numbers, size and glycoprotein content.	No change.
Late	Signet ring cell formation.	No change.
(f) Minor oestrogen excess.	Total loss of glycoprotein. Cell regression.	No change.
(g) Gross oestrogen excess.	Total loss of glycoprotein.	Loss of glycoprotein. Cell regression.

In the light of this functional specificity, the distinction of appearance and of distribution of the two types of cells assumes greater significance. We infer from these observations that the two types of glycoprotein producing cells are morphologically distinct and are stimulated by individual mechanisms to produce their own specific hormones. There is no evidence that one type of cell changes over to the other type in order to produce a different hormone when the hormonal demand is altered. One important piece of evidence for the complete individuality of these two cell types is the appearance seen in pituitaries of animals castrated and thyroidectomized. In these pituitaries thyroidectomy and castration cells are both present and retain their individual characteristics even when existing side by side. Castration cells are characterized by a high glycoprotein content and the hyaline filled vacuoles are surrounded by a thick

rim of cytoplasm containing a dense accumulation of glycoprotein granules. This produces the true signet ring appearance. One the other hand, the thyroidectomy cells are low in glycoprotein content and often contain multiple hyaline filled vacuoles. The vacuoles are enclosed by a thin, almost imperceptible, rim of cytoplasm which contains either none or only a few scattered glycoprotein granules. They do not, therefore, have a true signet ring appearance. Reese, Koneff and Wainman (1943) have pointed out the easy distinction between these two types of cells as seen by the Mallory-Azan type of staining and, as will be discussed below, the Mallory-Azan type of staining reveals the same cytological details as the PAS staining in these cell types. Halmi (1950), using the aldehyde fuchsin stain of Gomori (1950), differentiated two types of "basophil" cells which he calls beta and delta cells in the rat pituitary. From the nature and the distribution of these cells described by Halmi we think that his beta cells are the "thyrotrophs" and the delta cells are the "gonadotrophs." The sex difference in the number of delta cells and their response to castration makes their identification with our "gonadotrophs" reasonably certain, but the position of the beta cells is not so clear. In Halmi's observations these were described as being reduced in numbers after thyroidectomy, the increase in the so-called "basophils" after thyroidectomy being ascribed to an increase in delta cells. We think that this result may be due to the fact that the aldehyde fuchsin reveals the thyrotrophic hormone granule content of the cells and that when the hormone content is reduced in the "thyrotrophs," as it is after thyroidectomy, the absence of the aldehyde fuchsin staining has led Halmi to identify the thyroidectomy cells as delta cells. If this be the explanation Halmi's observations would be brought into line with ours.

(2) *Relationship of the glycoprotein, demonstrated histologically, to the hormone content of the gland.* When the glycoprotein, demonstrated histologically in the two types of cells distinguished above, is correlated with the known responses of the hormone content of the pituitary as determined by assay, it is found that the glycoprotein reaction in the gonadotrophic cells is correlated with the gonadotrophic hormone content, while the glycoprotein content of the thyrotrophic cells is correlated with the thyrotrophic hormone content. The correlation is illustrated in Table II in which are shown the glycoprotein responses and the parallel changes in hormone content as determined by assay. The classification of glycoprotein reaction as normal is based on the examination of a number of slides, but as cell counts were not made and as there is no exact method for measuring the amount of glycoprotein material in individual cells, deviations from normal of 20 or 30 per cent might go undetected. In every case, however, where the results of assay would indicate that the hormone

content was absent or considerably increased, the corresponding change in the glycoprotein was immediately obvious. No account has been taken in this table of the late term responses in either glycoprotein reaction or hormone content as the significance of such changes is at present obscure.

TABLE 2. RELATION OF GLYCOPROTEIN REACTION TO HORMONE CONTENT OF PITUITARY AS MEASURED BY ASSAY

Treatment of animal	Glycoprotein in gonadotrophs	Gonadotrophic hormone potency	Glycoprotein in thyrotrophs	Thyrotrophic hormone potency
Thyroxine deficiency	Normal	Normal (1)	Decreased	Decreased (2)
Thyroxine excess	Normal	Normal (3)	Absent	Absent (4)
Gonadal deficiency	Greatly increased	Greatly increased (5)	Little change	Little change (6)
Oestrogen excess:				
Small doses	Diminished	Diminished (7)	Normal	Normal (8)
Large doses	Absent	Absent (9)	Diminished	Diminished (10)

References

(1) Smelser (1939).
(2) Griesbach and Purves (1943).
(3) Griesbach and Purves (unpublished).
(4) Griesbach and Purves (1946).
(5) Engle (1929).
(6) Bomskov and Schneider (1939).
(7) Meyer, Leonard, Hisaw and Martin (1940).
(8) Shumacker and Lamont (1935).
(9) Griesbach and Purves (unpublished).
(10) Simpson and Evans (1941).

One type of PAS positive material in the pituitary cells which cannot be correlated with any hormone activity is the material forming coarse densely staining droplets which appear in chronic thyroxine deficiency in the thyrotrophic cells. These droplets, which are the most strongly staining elements to be found by the PAS method in the pituitary, are almost certainly the droplets observed by Catchpole in certain cells of the pituitary of the thyroidectomized rat. Catchpole was unable to relate these to the thyrotrophic hormone since the solubility of these droplets in frozen dried material was unlike that of the thyrotrophic hormone. In unfixed material we have found that these droplets are resistant to extraction by buffers which would extract the thyrotrophic hormone and which do extract the glycoprotein of the "thyrotrophs" of the normal pituitary. These droplets also differ from the normal glycoprotein in that they are not revealed by the Mallory type of staining as will be referred to later. They therefore differ chemically both from the normal glycoprotein of the "thyrotrophs" and from the thyrotrophic hormone as isolated from the pituitaries of larger animals. Their significance is therefore at present obscure. The meaning of the glycoprotein reaction in the normal pituitary cells, however, seems to be definitely established and we are satisfied that the glycoprotein in the cells which we call "gonadotrophs" represents gonadotrophic hormone and the glycoprotein in the cells which we call "thyrotrophs" represents the thyrotrophic hormone. The question whether the glyco-

protein of the gonadotrophic cells represents both the follicle stimulating and luteinizing hormone or whether it represents follicle stimulating hormone alone is discussed further in a later section.

(3) *Relationship of glycoprotein granules to cyanophils and basophils.* Since the evidence we have reviewed indicates that the PAS reaction demonstrates the actual sugar containing hormones in the specific cells which produce them, the PAS reaction gives a more fundamental distinction of cell types than that obtained by the use of other histological and cytological methods. It seems, therefore, that the discussion of the relation of the PAS positive cells to the cyanophils or basophils demonstrated by other methods is a matter of deciding what the staining reactions of the glycoprotein cells are by other methods. We have determined the staining reaction of the glycoprotein containing cells by our normal pituitary staining method (Mallory stain as modified by Martins). We found that the identical picture produced by the Schiff reaction in the PAS staining was obtained by the aniline blue staining of our modified Mallory technique. This correspondence extends down to the individual granules in the cytoplasm of the normal cells, but breaks down with the coarse droplets which appear in the "thyrotrophs" in long term thyroxine deficiency. We find by our method that the cells with the strongest PAS reaction are stained the darkest by the aniline blue. This result is a direct contrast to Catchpole's findings, namely, "these cells which contained larger amounts of glycoprotein were less "basophilic," and vice versa." We think it likely that this discrepancy is due to differential staining of the thyrotrophic and gonadotrophic hormones. In Azan stained sections, for instance, the "thyrotrophs" are stained a more intense blue than the "gonadotrophs" while the PAS reaction is stronger in the "gonadotrophs" than in the "thyrotrophs." Nevertheless, the aniline blue still stains the same elements as the PAS method demonstrates and all the cytological features and functional responses that are described in this paper for the two types of cells should be observable by the Mallory or Azan techniques. That this is so appears to be demonstrated by certain observations already published. In particular, Reese, Koneff and Wainman, in discussing the castration and thyroidectomy basophils, clearly have observed the two types of cells and have also observed a difference in their functional responses. We quote, "In discussing this point it is necessary to stress the difference between the basophils of normal female and male rats. By this criterion we believe one can differentiate pituitaries from the two sexes. The predominant type of basophil in the normal pituitary of the male is an oval or rounded cell which stains comparatively lightly; in the female the predominating basophil is polygonal in shape and more basophilic, and in this cell the mitochondria are large and usually spherical. In

the early response to thyroidectomy of either sex, a marked increase in number of basophils occurs, the cells closely approximating the type of cell just described for the normal female. They are irregular in shape, often polyhedral and have a distinctly increased basophilia. As Guyer and Claus have stated, thyroidectomy basophils show a marked tendency towards grouping. Castration basophils, on the other hand, in early stages of formation are compact cells which are oval or round. The granules are evently distributed and the cell stains as a rule more lightly with aniline blue than young thyroidectomy basophils." It will thus be seen that these workers have seen the two types of cells and have noticed the functional responses of the polyhedral type to thyroxine deficiency and of the oval type to gonadal deficiency. The important additional feature which we have observed is that the two types of cells are not characteristic one for each sex, but that both types of cell occur in both sexes. The "gonadotrophs," however, are so scanty in the female and are present for only a short time during the oestrus cycle that it is almost certain that they would be overlooked unless attention was specially directed to their detection. Moreover, the zonalization of these cells makes it easy to miss them, particularly if the sections are cut through the region in which they are normally scanty.

The term basophil as applied to the cells of the anterior pituitary derives from the fact that basic dyes applied to the pituitary of certain species, notably man, demonstrate certain cells with a relatively intense basophilic staining of the cytoplasm. This basophilic staining is due, apparently, to the presence of large amounts of ribonucleic acid as it is removed by ribonuclease. Ribonucleic acid is probably present in the cytoplasm of all the pituitary cells but the basophils have such large amount compared with the other types that they may be differentiated by this method. The basophilic cells of the human pituitary are also stained blue by the aniline blue of the Mallory and Azan techniques and these techniques have come to be regarded as an alternative method of demonstrating basophils. However, we would regard it as proper to regard the aniline blue staining as revealing glycoprotein. The correspondence between the basophilic cells of the human pituitary and the cyanophilic cells probably results from the fact that the glycoprotein producing cells in the human pituitary are predominantly those with the increased amount of ribonucleic acid revealed by ordinary basic stains. On this question the observations of Pearse (1949) on human pituitaries should be cited. Pearse found ahat PAS positive granules were in the basophil cells of the human pituitary. After treatment with ribonuclease the basophilic staining was absent, but the PAS reaction was still positive. The PAS reaction was, therefore, due

to a different substance from that responsible for the basophilic staining, namely, glycoprotein as compared with ribonucleic acid.

(4) *Localization of site of production of individual hormones.* The three pituitary hormones which contain sugar in significant amounts are the follicle stimulating and luteinizing hormones and the thyrotrophic hormone (Li and Evans, 1948). We have tested a purified thyrotrophic hormone preparation and find that it gives a positive PAS reaction. The functional responses of the glycoprotein in the two types of cells considered in conjunction with the results of assays indicate that the thyrotrophic hormone is formed by that section of the cyanophils which we called "thyrotrophs." Smelser (1944) assayed the central and peripheral zones of the bovine anterior pituitary gland for the thyrotrophic, adrenotrophic and gonadotrophic hormones. Although these hormones were found in greater concentration in the "basophilic" central zone, the ratios of centre:cortex were not the same for the three hormones. From this observation he drew the conclusion that the thyrotrophic, adrenotrophic and gonadotrophic hormones of the beef pituitary gland are produced or stored by several distinct cell types which have different spatial distributions in the gland. It is noteworthy that the highest ratio of centre:cortex content was found for the thyrotrophic hormone, the ratio being 16 or 32:1.

As regards the gonadotrophic hormone, it would appear certain that the follicle stimulating hormone is produced by the other type of cyanophil which we called "gonadotrophs." The site of production of the luteinizing hormone is not, however, established. One reason for this is the lack of suitable assay methods for the luteinizing hormone. Furthermore, the conditions which depress the formation of the gonadotrophic hormones, i.e. oestrogen administration, lead to a loss of both hormones from the pituitary. We did not, therefore, have an opportunity of examining the PAS reaction of pituitary which contained luteinizing hormone, but no follicle stimulating hormone. However, the absence of any important glycoprotein reaction from the cytoplasm of the acidophils seems to us to exclude them as a site of production of the luteinizing hormone. The cytoplasm of the acidophils gives a faint glycoprotein reaction as does cytoplasm of the chromophobes, but this slight reaction is probably due to sugar content in the structural proteins of the cell. It does not show any fluctuation with variation in the amount of gonadotrophic hormone as induced by oestrogen administration or gonadal deficiency. Catchpole (1948) discusses the possibility of FSH and LH being present in the same cells and finds that the granules of these cells (the "gonadotrophs") were partially soluble in 0.25M acetate buffer at pH 4.4 containing 20.5% sodium sulphate. Al-

though we have not sufficient evidence to show that luteinizing hormone of the rat would be insoluble in such a buffer mixture, the fact that only partial extraction was obtained by Catchpole with this solution would indicate the presence of two glycoproteins in the "gonadotrophs." It, therefore, seems likely that both the follicle stimulating and luteinizing hormones are produced in the same cell types, namely, the gonadotrophic cells.

The characterized hormones of the pituitary other than the gonadotrophic and thyrotrophic hormones do not contain a significant amount of sugar and the PAS reaction, therefore, gives no evidence about the sites of formation of these other hormones. However, the apparent complete inactivity of the "gonadotrophs" and the "thyrotrophs" under conditions when the production of the glycoprotein hormones is inhibited, i.e. with oestrogen and thyroxine administration respectively, seems to indicate that these cells have no other function than the production of glycoprotein hormone. It would therefore seem unlikely that any other non-glycoprotein hormone will be found to be produced by these cells. Also there does not seem to be any evidence for the view that any specific cell type can be switched from the production of one hormone to the production of another according to the hormonal demands of the animal. To what extent this individuality will prove to be true of the remaining cells and hormones of the anterior pituitary must await the introduction of differential staining methods which will subdivide the group of cells classed as chromophobes. There is no evidence that these represent a homogeneous cell type. Their classification as chromophobes merely indicates that they do not have the large concentration of acidophilic elements that characterize the acidophils and are free from the glycoprotein granules to which is due the staining reaction by which the socalled cyanophils or "basophils" of the rat's pituitary are differentiated. Moreover, it should be remembered that in certain animals two types of acidophils can be readily distinguished by the Mallory-Azan technique and that even in animals in which two types of acidophils cannot be differentiated there still remains the possibility that the acidophil group comprises more than one type of cell. It is thus still possible that most of the hormones of the anterior pituitary will eventually be found to be the product of specific cell types.

SUMMARY

The results of the McManus periodic acid Schiff reagent (PAS) staining for glycoprotein applied to sections of the rat pituitary have been correlated with the results of Mallory staining and with the hormone content of the pituitary determined by assay. It was found that glycoprotein granules

present in the cytoplasm are responsible for the blue staining of the "basophils" by aniline blue in the Mallory technique. The socalled "basophils" are, therefore, glycoprotein producing cells.

Two types of glycoprotein containing cells are distinguishable. One type is an oval or rounded cell staining intensely by PAS and localized to the lower surface of the anterior pituitary and to the upper surface adjacent to the pars intermedia. This type is inhibited by oestrogen and is the source of castration cells in gonadal deficiency. The PAS reaction in these cells correlates with the known gonadotrophic hormone content of the gland under experimental conditions. It is concluded that this type is the site of production of gonadotrophic hormone and the name "gonadotrophs" is suggested for these cells.

The second type of glycoprotein containing cells is polyhedral in shape and occupies the central region of the anterior lobe. This type is inhibited by thyroxine administration and responds to thyroxine deficiency by producing thyroidectomy cells. The intensity of the PAS reaction in these cells is correlated with the thyrotrophic hormone content of the gland. It is concluded that these cells, for which the name "thyrotrophs" is suggested, are responsible for the production of thyrotrophic hormone.

It is considered unlikely that either of these cell types, which together constitute the socalled "basophils," are responsible for the production of any hormones other than the specific glycoprotein hormones from which their suggested names are derived.

REFERENCES

Adams, A. E.: *Quart. Rev. Biol.* **21:** 1. 1946.

Bomskov, C. and E. Schneider: *Arch. exp. Path. Pharmak.* **191:** 715. 1939.

Catchpole, H. R.: *Endocrinology* **6:** 218. 1949.

Engle, E. T.: *Am. J. Physiol.* **88:** 101. 1929.

Glick, D.: Techniques of Histo- and Cytochemistry, p. 67. Interscience Publishers, New York. 1949.

Gomori, G.: *Am. J. Clin. Path.* **20:** 665. 1950.

Griesbach, W. E.: *Brit. J. Exp. Path.* **22:** 245. 1941.

Griesbach, W. E. and H. D. Purves: *Brit. J. Exp. Path.* **24:** 174. 1943.

Griesbach, W. E. and H. D. Purves: *Brit. J. Exp. Path.* **26:** 13. 1945.

Halmi, N. S.: *Endocrinology* **47:** 289. 1950.

Hotchkiss, R. D.: *Arch. Biochem.* **16:** 131. 1948.

Li, Ch. H. and H. M. Evans: The Hormones, Vol. 1, p. 631. Academic Press, New York. 1948.

McManus, J. F. A.: *Nature,* London. **158:** 202. 1946.

Martins, T.: *C. R. Soc. Biol.* Paris. **113:** 1275. 1933.

Meyer, R. K., S. L. Leonard, F. L. Hisaw and S. J. Martin: *Proc. Soc. exp. Biol.* New York. **27:** 702. 1930.

Pearse, A. G. E.: *J. of Path. and Bact.* **61:** 195. 1949.

PURVES, H. D. AND W. E. GRIESBACH: *Brit. J. Exp. Path.* **27:** 170. 1946a.
PURVES, H. D. AND W. E. GRIESBACH: *Endocrinology* **39:** 274. 1946b.
REESE, J. D., A. A. KONEFF AND P. WAINMAN: Essays in Biology, p. 473. Univ. of Calif. Press. 1943.
SALTER, W. T.: The Hormones, Vol. I. p. 305. Acad. Press. New York. 1950.
SEVERINGHAUS, A. E.: *Physiol. Rev.* **17:** 556. 1937.
SHUMACKER, H. B. AND A. LAMONT: *Proc. Soc. exp. Biol.* **32:** 1568. 1935.
SIMPSON, M. E. AND H. M. EVANS: *Anat. Rec.* Suppl. 2. **79:** 57. 1941.
SMELSER, G. K.: *Anat. Rec.* **74:** 7. 1939.
SMELSER, G. K.: *Endocrinology* **34:** 39. 1944.

HISTOCHEMICAL DEMONSTRATION OF KETOSTEROIDS IN NORMAL AND NEOPLASTIC TESTES[1]

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A METHOD for the histochemical demonstration of active carbonyl groups of lipoid in various endocrine glands such as adrenal cortex, ovary, testicle, and placenta has been described elsewhere (Ashbel and Seligman, 1949; Seligman and Ashbel, 1949; Seligman, Friedman and Herz, 1949). Normal and neoplastic nervous tissue (Seligman and Ashbel, 1951), adrenal cortical tumors associated with precocious development and virilism (Seligman and Ashbel, 1951b), and adrenal cortical tumors with and without Cushing's syndrome (Seligman and Ashbel, in preparation) have been studied with this method. Supplementary reactions which demonstrated the active carbonyl groups to be ketonic in character and not aldehydic (Seligman and Ashbel, 1951a and b) have been performed regularly. Therefore, whenever lipoidal ketones are demonstrated in tissue known to contain ketosteroid, it is presumed although not conclusively proved that ketosteroids are responsible for the reaction.

This communication presents the results of a study of the localization of ketosteroids in the testes of different species of animals in normal and in some pathological conditions. Included is a short series of interstitial and sertoli cell tumors of the dog, several canine and human seminomas, and three experimentally produced interstitial cell tumors of the mouse.

METHOD

Frozen sections, 10–20 microns thick, were made from tissues which had been fixed in neutral 10% formalin for a few hours to as long as several years. The sections were mounted on glass slides, dried for a few minutes, and then washed for 2 hours in several changes of water to remove the formalin. Some sections from each block of tissue were stained for fat with sudan IV. Others were stained for active carbonyl groups by the

Received for publication May 14, 1951.

[1] This investigation was supported by a research grant from the National Cancer Institute, National Institutes of Health, Public Health Service, Federal Security Agency.

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method described by us earlier with the reagents,[4] 3-hydroxy-2-naphthoic acid hydrazide and tetrazotized diorthoanisidine. In order to determine whether carbonyl containing material was lipoidal in nature, other sections were dehydrated in stages of 30 minutes utilizing 70%, 95% and 100% alcohol in that order, and then were immersed in absolute alcohol, xylol, alcohol-xylol, chloroform, acetone, pyridine or absolute ether for 24 hours. They were then removed and rehydrated, utilizing 100%, 95% and 70% alcohol successively and finally water. The sections were then stained for active carbonyl groups.

In addition, the procedures described earlier to prove the ketonic character of the carbonyl groups in the lipoid of formalin fixed tissues, were repeated in representative types of each group of cases. The carbonyl groups demonstrated in this study were found in lipoid material and were ketonic and not aldehydic. Other sections prepared from paraffin embedded tissue were stained with hematoxylin-eosin.

In order to distinguish between estrogenic ketosteroid (estrone) and the androgenic ketosteroids, advantage was taken of the phenolic character of the 3-hydroxyl group in the aromatic ring A of estrone, which was extracted from tissue by incubation in alkaline solution at about pH 11 prior to testing for ketosteroid. They were placed in 50 cc. of either 0.5% potassium carbonate solution or 0.5% trisodium phosphate solution for 24 hours. Control sections were immersed in 50 cc. of distilled water for the same time. Both types of sections were then washed in distilled water for several hours and stained for ketonic carbonyl groups.

Black and white photomicrographs of sections stained for ketosteroids were taken through a red filter and sections stained with sudan IV were photographed through a green filter.[5]

Data on the quantity of sudanophilic material and the intensity of the reaction for lipoidal ketonic carbonyl groups (ketosteroid) presented in tables 1–5 were obtained as an estimate, by the inspection of many slides of each specimen by two independent observers. Absence of either stain is denoted by the symbol (−). Positive reactions are designated as follows: a small amount of staining in isolated foci (+), moderate to intense staining in many cells with large numbers of unstained cells (++), slight to moderate staining in nearly all cells (+++), and intense staining throughout the sections (++++).

RESULTS

I. *Normal animals*

The testes of frogs, roosters, mice, rats, rabbits, pigs, dogs, horses, and a goat were examined for lipoid and lipoidal ketonic carbonyl groups (ketosteroid). The results are summarized in tables 1 and 2.

Animals with seasonal breeding periods show seasonal variations in the histology of the testis as well as in hormonal pattern (Aron, 1921; 1925; Benoit, 1925; Champy, 1908; Humphrey, 1921; Wislocki, 1949). In frogs obtained at the end of July, the interstitial cells contained moderate quantities of ketosteroid which stained as fine blue granules, whereas the sertoli cells were rarely stained a faint blue color. Sudanophilic droplets

[4] All reagents for this test may be obtained from Dajac Laboratories, Monomer-Polymer, Inc., 511 Lancaster Ave., Leominster, Mass.

[5] Photomicrographs by Mr. Leo Goodman.

TABLE 1. KETOSTEROID AND LIPOID IN TESTIS OF NORMAL ANIMALS

Specie	Experiment No.	Age	Ketosteroid reaction		Sudanophilia	
			Sertoli cell	Interstitial cell	Sertoli cell	Interstitial cell
Frog	554	adult	−	++	++	+++
Frog	555	adult	−	++	++	+++
Frog	556	adult	−	++	++	++
Frog	557	adult	−	+++	++	+++
Frog	558	adult	+	+++	++	+++
Rooster	575	adult	−	−	−	++
Rooster	576	adult	−	−	−	++
Mouse[1]	548	young	−	+++	−	+++
Mouse[2]	551	old	−	++	−	++
Rat	123	adult	−	++	−	++
Rat	124	adult	−	++	−	++
Rabbit	120	adult	−	++	−	++
Rabbit	125	adult	−	++	−	++
Rabbit	132	adult	−	++	−	++
Horse	476	adult	−	+++	−	+++
Horse	490	5 yrs.	−	+++	−	+++
Goat	494	adult	−	+++	−	+++
Pig	607	6 weeks	+++	++++	+++	++
Pig	607/A	6 weeks	+++	++++	+++	++
Pig	607/B	6 weeks	++	+++	++	++

[1] Estimation based on examination of six animals.

were moderate to abundant in both types of cells. Ketosteroid was not demonstrable in either type of cell in two roosters taken in August. The interstitial cells contained lipoid, however.

In the mouse, rat, rabbit, horse and goat, ketosteroid and lipoid were demonstrable in the interstitial cells, but neither was seen in the sertoli cells. On the other hand, the testes of three pigs six weeks of age contained

TABLE 2. KETOSTEROID AND LIPOID IN TESTIS OF NORMAL DOGS

Experiment No.	Hospital No.	Age	Ketosteroid reaction		Sudanophilia	
			Sertoli cell	Interstitial cell	Sertoli cell	Interstitial cell
108		adult	−	++++	−	++++
119		adult	−	++++	−	++++
546		adult	−	++++	−	++++
233		adult	−	++++	−	++++
228		adult	−	++++	−	+
230		adult	−	++++	−	++++
570		adult	−	+++	−	+++
482[1]	(850-133)	1.5 yrs	−	++++	−	++++
486[1]	(849-180)	adult	−	++++	−	++++
483[1]	(849-164)	adult	+	++++	++	++++
485[1]	(850-134)	10 years	+	++++	++	++++

[1] Provided through the courtesy of the Angell Memorial Animal Hospital, Boston.

a proportionately larger number of interstitial cells than were seen in the other animals. They contained fine granules and droplets of ketosteroid which reacted intensely. The sudanophilic stain was less intense than the ketosteroid reaction and was finely distributed in faintly staining granules throughout the cytoplasm. The sertoli cells of the pigs were unusual not only in their lipoid content, but in the strong reaction for ketosteroid (fig. 1).

The results of examination of the testes of 11 adult dogs are given in table 2. Seven of these dogs had died in the course of experiments on hemorrhagic shock. Whereas the interstitial cells of all the dogs contained ketosteroid and lipoid in large quantities, the sertoli cells in only two dogs (483 and 485) contained a small quantity of both lipoid and ketosteroid in focal portions of some sections.

FIG. 1. Normal testis of 6 weeks old pig (607). The tubules are separated by masses of polygonal interstitial cells whose cytoplasm are filled with deep blue granules. The sertoli cells also contain these blue staining cytoplasmic granules in considerably smaller quantities. The nuclei do not contain this material. Ketosteroid reaction. ×200.

FIG. 2. Interstitial cell tumor of dog (492). The granular distribution of ketosteroid in the cytoplasm of tumor cells is shown. Ketosteroid reaction. ×600.

FIG. 3. Interstitial cell tumor of dog (151). Cords of interstitial cells show sudanophilic material in the form of large and small droplets filling the cell cytoplasm and frequently displacing the nucleus. Sudan stain. ×180.

FIG. 4. Interstitial cell tumor of dog (151). Interstitial cell cytoplasm is filled with blue granular material which is occasionally clumped but in most instances is diffusely dispersed throughout the cytoplasm. This appearance is uniform throughout the tumor. Ketosteroid reaction. ×180.

FIG. 5. Interstitial cell tumor of dog (150). Large globules of sudanophilic material fill the cytoplasm of the tumor cells. Occasional pale or unstained vacuoles are seen. Sudan stain. ×200.

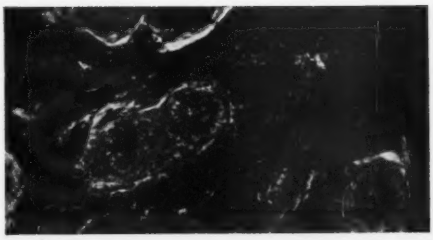
FIG. 6. Interstitial cell tumor of dog (150). Large globules of ketosteroid occupy large portions of the cytoplasm of tumor cells. This appearance is uniform throughout the tumor. Ketosteroid reaction. ×200.

FIG. 7. Sertoli cell tumor of dog (480). Globules of sudanophilic material are present in many of the tumor cells. Occasionally the stained material concentrates at the periphery of large vacuoles to form a demilune. Sudan stain. ×180.

FIG. 8. Sertoli cell tumor of dog (480). The tubular pattern of this tumor is apparent. Many areas are entirely free of ketosteroid. Large droplets of ketosteroid are seen in the cytoplasm of tumor cells in focal parts of the section. Ketosteroid reaction. ×180.

FIG. 9. Human testis (633). Large droplets of sudanophilic material fill the cytoplasm of interstitial cells. Finer droplets are present in the cytoplasm of sertoli cells. Sudan stain. ×200.

FIG. 10. Human testis (633). A granular distribution of ketosteroid is noted in the cytoplasm of interstitial cells. The sertoli cells contain cytoplasmic granules which occur most frequently toward the basement membrane. Ketosteroid stain. ×200.



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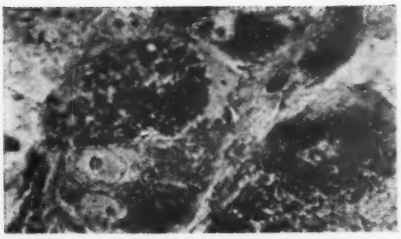

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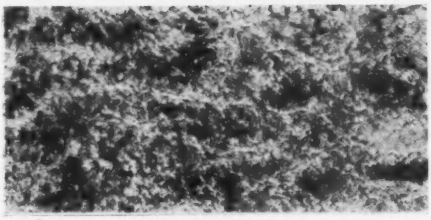

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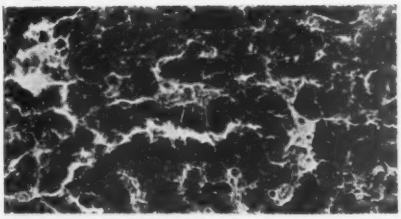

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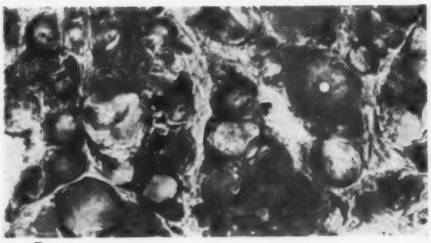

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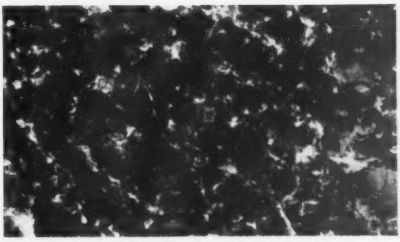

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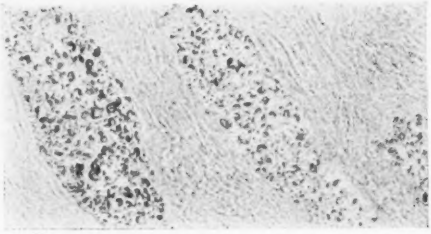

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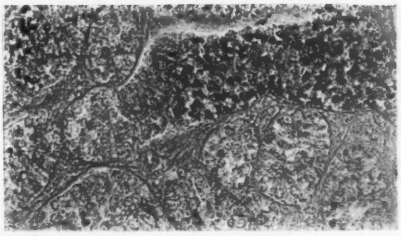

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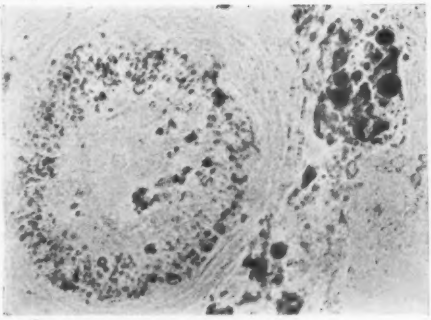

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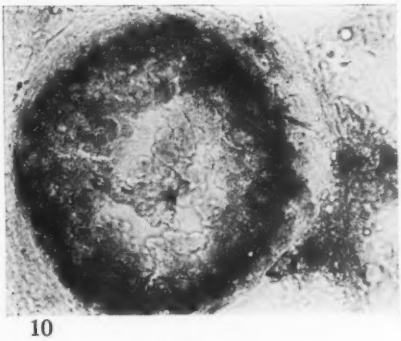

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TABLE 3. KETOSTEROID IN CRYPTORCHID TESTES[1] OF DOGS

Experiment No.	Hospital No.	Age, Years	Ketosteroid reaction		Sudanophilia	
			Sertoli cell	Interstitial cell	Sertoli cell	Interstitial cell
489	(A-49-53)	8	+	++++	+	++++
491/1	(A-50-41)	15	+++	++++	+++	++++
501	(S50-139)	adult	++	++++	++	++++
502/B	(A-50-141)	10.5	+++	++++	++	++++

[1] Provided through the courtesy of the Angell Memorial Animal Hospital, Boston.

II. *Cryptorchid and atrophic testes of the dog* (table 3)

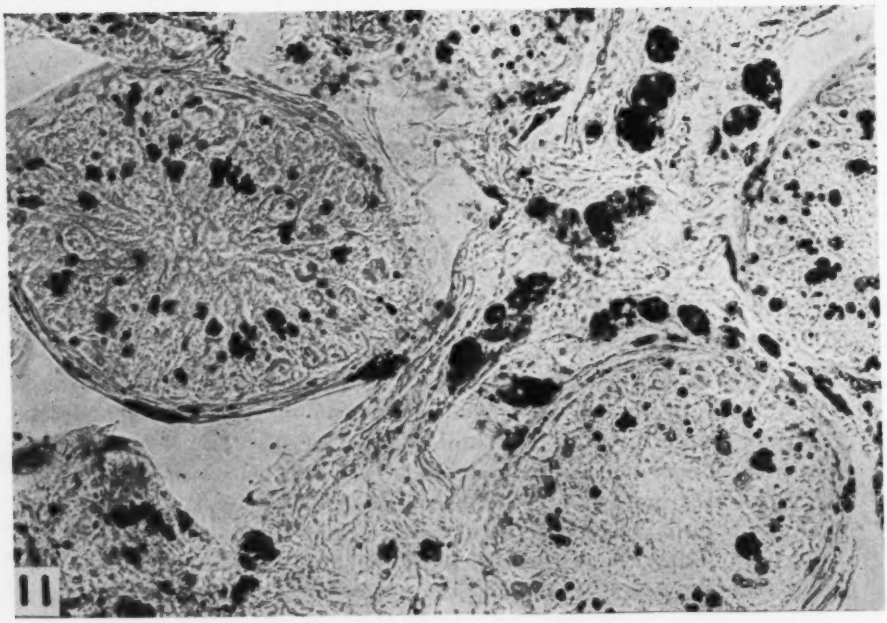
Three testes were cryptorchid; the fourth was atrophic but intrascrotal (489, table 3). In three there were associated tumors, a seminoma, a sertoli cell tumor, and a tumor of uncertain origin. The spermatic elements were completely atrophied. Sertoli and interstitial cells remained intact. The interstitial cells uniformly contained large quantities of ketosteroid and lipoid in the form of large and small droplets in the cytoplasm. In contrast to the normal dog, the sertoli cells in all four testes contained droplets of ketosteroid distributed throughout the cell cytoplasm, although in greater concentration in the central and luminal portions of the cell (figs. 11 and 12). The significance of the abundance of ketosteroid in these sertoli cells is not clear.

III. *Interstitial and sertoli cell tumors of the dog* (table 4)

Six interstitial cell tumors and 5 sertoli cell tumors were selected by examination of paraffin sections stained with hematoxylin-eosin, from a larger group obtained from several institutions, as the most typical histologic examples of their types. In several instances a seminoma was also present in another portion of the same testis. Tumors of doubtful histologic diagnosis were not included in this series. As far as is known, none of these tumors had metastasized at the time of operation. Predominant symptoms or signs as far as they were known are given in table 4.

The diagnosis of a sertoli cell tumor was based on the presence of tubular structure at least in portions of the tumor, and proper orientation of the cells to a basement membrane, as well as similarity of the tumor cells to sertoli cells (figs. 13 and 14). Sertoli cell tumors had a tendency to become undifferentiated in areas with loss of the diagnostic characteristics (portions of fig. 5). This tendency is probably responsible for the designation of "diffuse sertoli cell tumor" by Huggins and Moulder (1945). Tumors with a predominantly undifferentiated pattern were not included in this series.

The diagnosis of interstitial cell tumor depended on the similarity of the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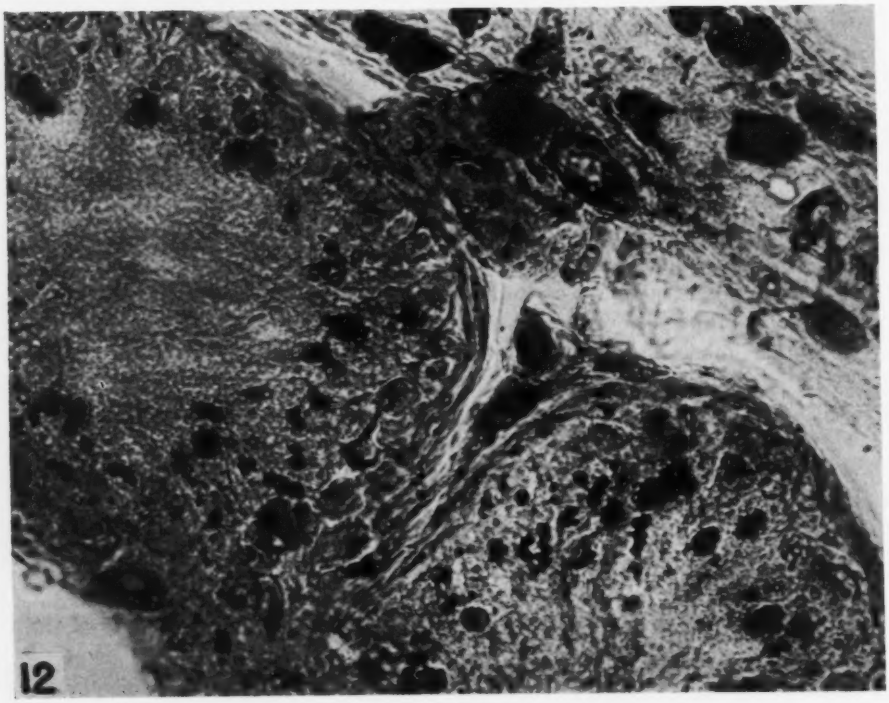
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TABLE 4. KETOSTEROID IN TESTICULAR TUMORS OF DOGS

Experiment No.	Hospital No.	Age, Years	Pathologic diagnosis	Sudan IV	Ketosteroid reaction	Predominant symptoms or signs
150[1]	(S48-139)	11	Interstitial cell tumor	++++	++++	weakness
151[1]	(S48-134)	12	Interstitial cell cell	++++	++++	bilateral perineal hernia
235[2]		adult	Interstitial cell tumor	+	+	
237[2]		adult	Interstitial cell tumor	++++	++++	
487[1]	(S49-141)	9	Interstitial cell tumor	++++	++++	large testis
499	(25709)	adult	Interstitial cell tumor	++++	++++	loss of hair and pigmentation of skin
503/B[1]	(A50-259)	15	Sertoli cell tumor	+++	++	senility and weakness
502[1]	(S50-151)	10.5	Sertoli cell tumor	++++	++++	perineal hernia and bilateral cataracts
480[1]	(S48-117)	8	Sertoli cell tumor	+++	+++	nipple cysts and pigmentation of skin
479[1]	(A48-243)	11½	Sertoli cell tumor	+	+	large testis
493[1]	(A50-235)	17	Sertoli cell tumor	++	++	large testis

[1] Provided through the courtesy of the Angell Memorial Animal Hospital, Boston.
[2] Provided through the courtesy of the Department of Anatomy, Yale University.

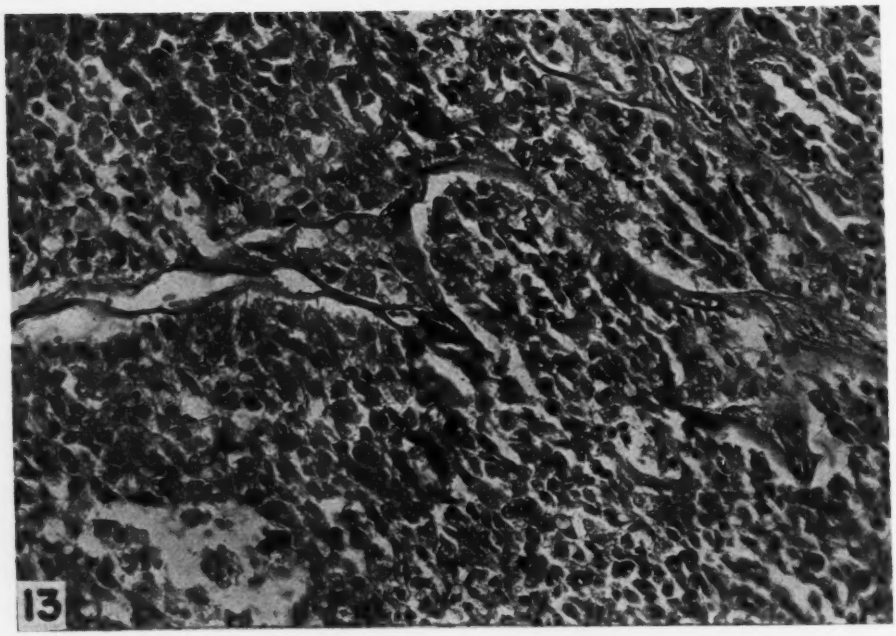
tumor cells to normal interstitial cells, and particularly upon their orientation to blood vessels (figs. 15 and 16).

The interstitial cell tumors contained large quantities of lipoid and ketosteroid, which were distributed in the cytoplasm of tumor cells as droplets or granules of varying size (figs. 2–6). Tumor 235 was exceptional. The sertoli cell tumors contained less ketosteroid, which was more focally distributed in the tumors thus leaving large areas unstained (figs. 7, 8, 17, 18). However, the two types of tumors could not be differentiated on the basis of the pattern of distribution of lipoid or ketosteroid.

A basis for differentiation was suggested by experiments which attempted to determine whether the ketosteroids in these tumors were androgenic or estrogenic. Since the latter is alkali soluble, prior extraction of the sections with sodium carbonate or trisodium phosphate would be expected to result in blocking of the ketosteroid reaction for estrone but not for the androgens. Alkaline extraction did not affect the ketosteroid

FIG. 11. Cryptorchid testis of dog (491/1). The cytoplasm of the interstitial cells contains large droplets of sudanophilic material, which frequently occupies almost the entire cytoplasmic area. In some cells there is a finer granular distribution. The sertoli cells contain cytoplasmic droplets of sudanophilic material with a tendency to localize in the luminal portion of the cell. Sudan stain. ×400.

FIG. 12. Cryptorchid testis of dog (491/1). The distribution of ketosteroid is similar to the distribution of sudanophilic material shown in fig. 11. Ketosteroid reaction. ×760.



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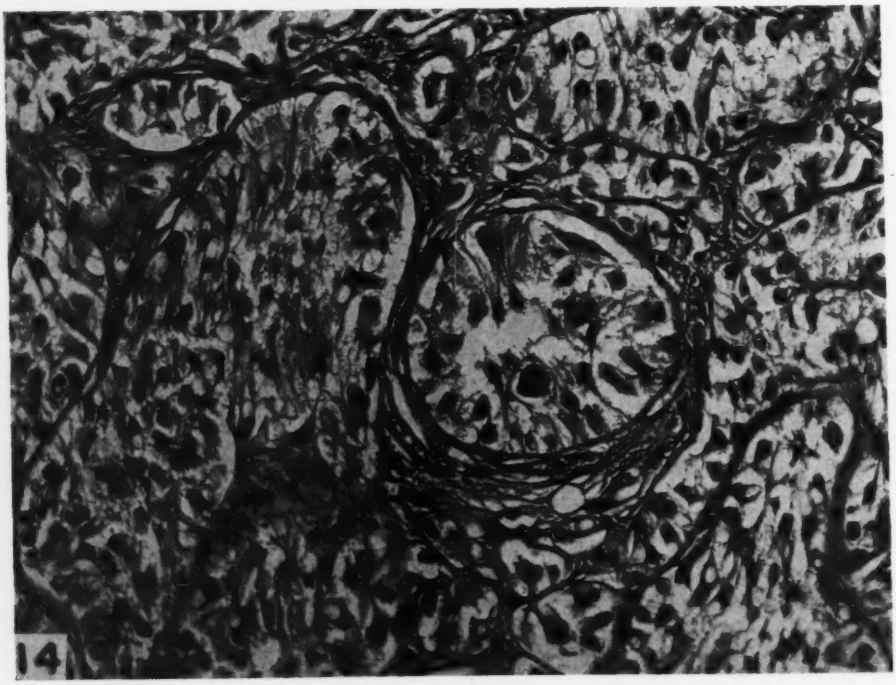

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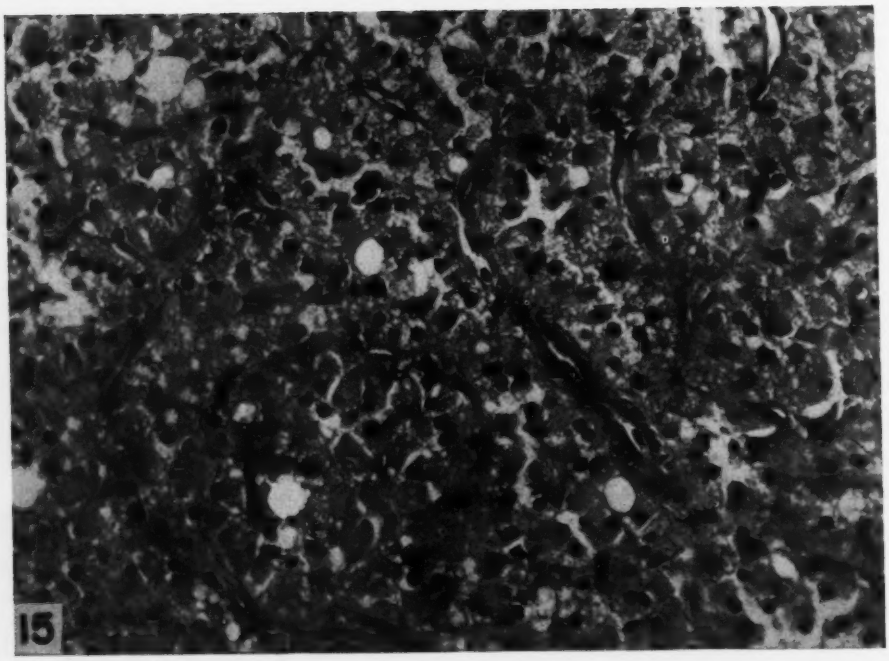
TABLE 5. KETOSTEROID IN HUMAN TESTIS[1]

Experiment No.	Age (yrs.)	Cause of death or surgical procedure	Ketosteroid reaction		Sudanophilia		Spermatogenesis
			Sertoli cell	Interstitial cell	Sertoli cell	Interstitial cell	
624	newborn		−	−	−	−	0
607	5	acute leukemia	−	−	−	−	0
603	5	pneumonia	−	−	−	−	0
604	7	pneumonia	−	−	−	−	0
602	10	pneumonia	−	−	−	−	0
600	17	rheumatoid arthritis	−	+	−	++++	active[2]
608	20	virus pneumonia	+++	++	++	++++	active
623	34	acute lymphatic leukemia	++	+	++	++	active
634	41	diabetes mellitus	+	+++	+++	+++	active
631	47	syringomyelia	+	++++	++++	++++	active
632	49	carcinoma of liver widespread metast	++++	+	++++	++++	active
633	49	acute hepatitis	++++	+	++++	+++	active
593	54	carcinoma of lung-metastasis widespread	+++	+++	++++	+++	active
622	65	inguinal hernia	−	−	+	+	active
595	65	carcinoma prostate	++	++	+++	+++	depressed[3]
635	65	carcinoma prostate	−	−	+++	+++	depressed
596	69	carcinoma prostate	++	++	++++	++++	depressed
621	75	carcinoma stomach	+	−	+++	+++	depressed
581	78	carcinoma prostate	−	−	+++	+	depressed
597	78	carcinoma prostate	++	−	+++	+	depressed
598	85	carcinoma prostate	++	++	++	++	absent

[1] These specimens were provided through the courtesy of the departments of pathology, Beth Israel Hospital, the Children's Hospital, Boston, the Deaconess Hospital, Boston, the Boston City Hospital and the Massachusetts General Hospital.
[2] Suicide following ACTH therapy and development of Cushing's syndrome.
[3] Stilbesterol therapy.

FIG. 13. Sertoli cell tumor of dog (480). Same as figs. 7 and 8. The tumor consists of cords of cells which occasionally form pseudotubules. Many cells are oriented to a basement membrane. The nuclei are vesicular, contain nucleoli, and clumped chromatin. The cell cytoplasm is not well demarcated and frequently appears to form a syncytium with neighboring cells. There is considerable cytoplasmic vacuolation. Hematoxylin-eosin. ×360.

FIG. 14. Sertoli cell tumor of dog (493). Same as figs. 17 and 18. The tumor consists of nests and cords of polygonal shaped cells with vacuolated cytoplasm. These cell nests occasionally open centrally to form pseudotubules lined by a single, or several layers of cells oriented to a basement membrane. The nuclei are frequently hyperchromatic. Mitotic figures are present. Hematoxylin-eosin. ×400.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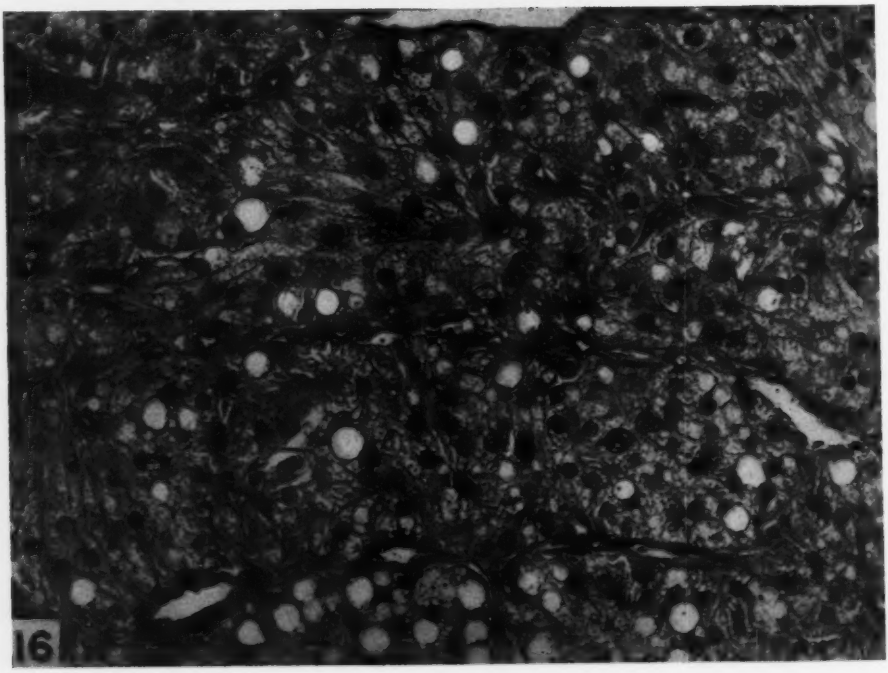
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reaction in three interstitial cell tumors but completely blocked it in three sertoli cell tumors. Furthermore, extraction at lower pH (sodium bicarbonate) did not block the reaction. This indicated that the acidic group was phenolic in character. These observations lend weight to the hypothesis that the reaction for lipoidal ketonic carbonyl groups demonstrates ketosteroid in these tissues. The procedure was less reliable in tumors which appeared to contain both types of cells because of the difficulty in recognizing partial removal of ketosteroid from the sections with certainty. The interstitial cells from four normal dogs were unaffected by alkaline extraction, but in two others (483 and 485) with a small amount of ketosteroid in the sertoli cells, they were bleached slightly along with the sertoli cells. The same was true of the interstitial cells of the testes of four humans (see below), of the three pigs, and in the cryptorchid dogs. Bleaching was not complete, on the other hand, in the sertoli cells of the 3 pigs and the cryptorchid dogs. It appears that some estrone may be present in interstitial cells and that the ketosteroid reaction of some sertoli cells is due to more than estrone.

In the several seminomas (dogs) examined, both lipoid and ketosteroid were absent.

IV. *Testes of human subjects* (table 5)

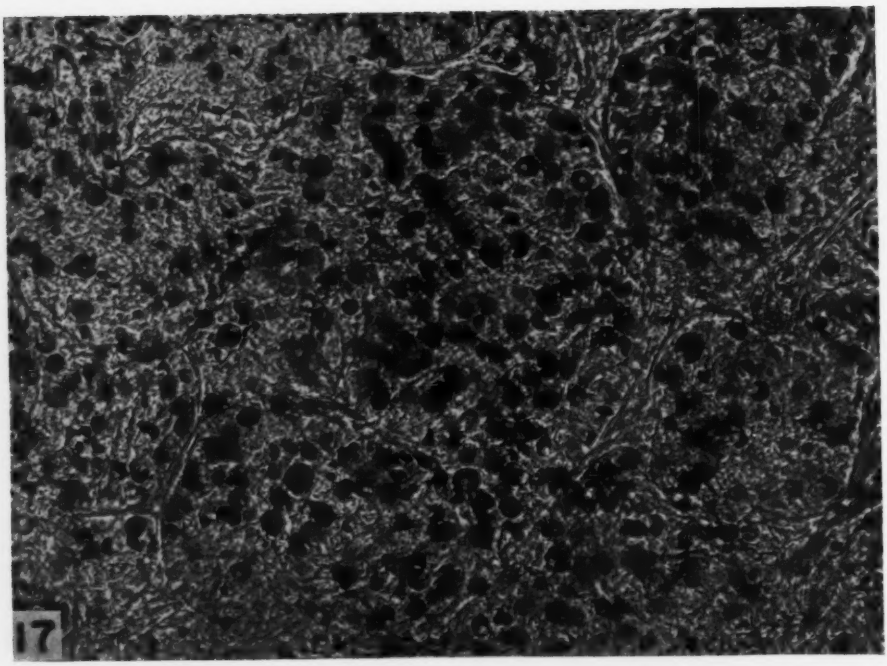
The testes of 21 subjects varying in age from newborn to 85 years were obtained as surgical or autopsy material from several hospitals and were examined for lipoid and ketosteroid. Presumably none can be considered completely normal since many came from patients dead after long illness, or from patients during treatment for carcinoma of the prostate, some after hormone therapy prior to orchidectomy.

Spermatogenic elements in all the testes contained neither lipoid nor ketosteroid. Furthermore, lipoid and ketosteroid were not found in the interstitial and sertoli cells of two human embryos (12 and 18 weeks of age) or of the five children included in table 5.

The interstitial cells of all but five adults contained variable quantities of ketosteroid. The largest amount was observed in those between 40–60

FIG. 15. Interstitial cell tumor of dog (151). Same as figs. 3 and 4. The tumor consists of cords of cells containing considerable eosinophilic cytoplasm and small hyperchromatic nuclei. The cells are oriented along blood vessels with their nuclei located at the poles of the cells farthest from the blood vessels. The cytoplasm is extensively vacuolated. No mitotic figures are present. Hematoxylin-eosin. ×400.

FIG. 16. Interstitial cell tumor of dog (150). Same as figs. 5 and 6. The tumor consists of cells oriented along blood vessels. The cytoplasm is extensively vacuolated with frequent large droplets which distend the cell. The nuclei are vesicular and contain clumped chromatin. No mitotic figures are present. Hematoxylin-eosin. ×400.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years of age, although moderate amounts were present in some of the older men. The interstitial cells of all the adults contained considerable sudanophilic material, even when there was little or no demonstrable ketosteroid.

The sertoli cells of all the adults contained considerable lipoid. The sertoli cells of scattered tubules in all but four adults contained varying amounts of ketosteroid as fine granules or small droplets. The largest amount of ketosteroid in sertoli cells was observed in two adults (632 and 633) with extensive liver damage (figs. 9 and 10). Both showed active spermatogenesis and rather low ketosteroid in their interstitial cells. The significance of these observations is not clear.

In six human seminomas, neither lipoid nor ketosteroid was demonstrable.

V. *Experimental interstitial cell tumors of mice*

Three interstitial cell tumors[6] which had been induced in the testes of strain A mice by administration of estrogen (14–17), and had been transplanted repeatedly into the subcutaneous space of other strain A mice, were found to stain intensely for lipoid and ketosteroid (figs. 19 and 20). One of these tumors was a thirty-fifth generation transplant.

DISCUSSION

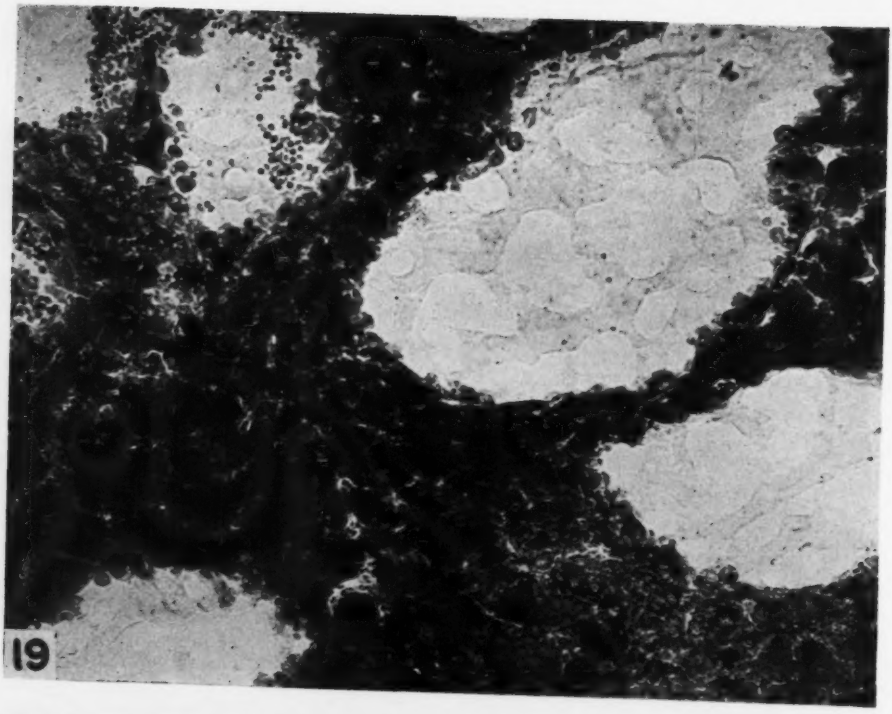
The role of the interstitial cell of the testis has been the subject of investigation for one hundred years. The anatomy of these cells was described by Leydig in 1850. Their physiologic function was demonstrated by various techniques including ablation-transplantation experiments, the production of experimental cryptorchidism, and observation of animals in various stages of development and sexual activity (Aron, 1929; Bouin and Ancel, 1903a, b, 1904a, b and c; Leydig, 1850; Romeis, 1933; Whitehead, 1904). It was generally concluded that the interstitial cells represented one of the sites of formation of the androgenic hormones which it was later found could be recovered from testicular tissue.

The sertoli cell was described in 1865 (Sertoli). Its function has never been adequately determined. It was first regarded as a supportive and nutritive cell. More recently the finding of lipoid droplets in these cells, plus evidence derived from a clinical syndrome of feminization associated

[6] Provided through the courtesy of Dr. W. U. Gardner, Yale University.

FIG. 17. Sertoli cell tumor of dog (493). Globules of sudanophilic material occupy portions of the cytoplasm of tumor cells. The semilunar pattern described in fig. 14 is present. Sudan stain. ×400.

FIG. 18. Sertoli cell tumor of dog (493). Nests of tumor cells contain large quantities of ketosteroid. Frequently there is a concentration of this material at the periphery of vacuoles. Ketosteroid stain. ×360.



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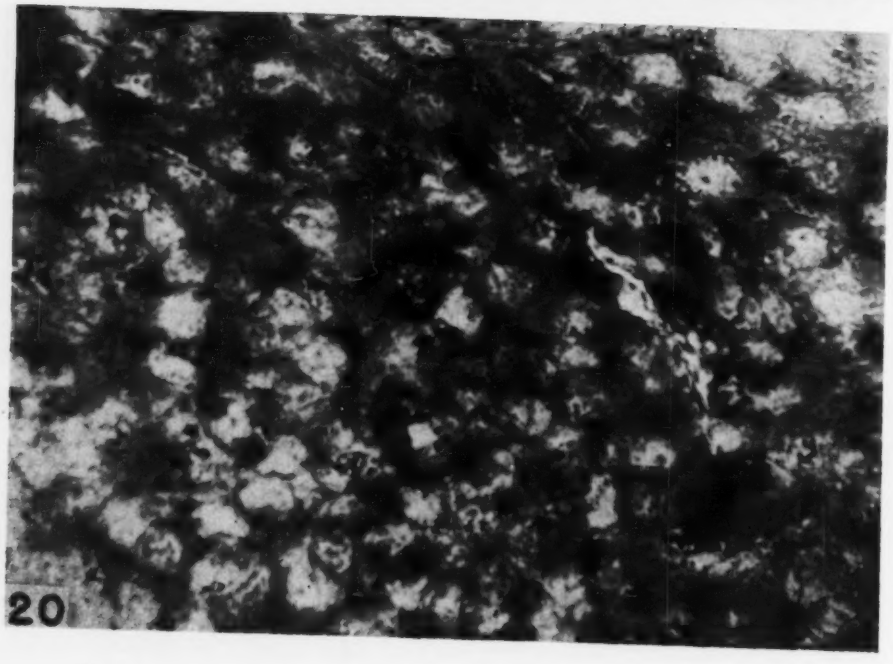

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with tumors supposedly arising from sertoli cells (Huggins and Moulder, 1945; Teilum, 1949, 1950), has suggested an endocrine function possibly connected with the production of estrogens. Huggins and Moulder (1945) have reported a high estradiol concentration by extraction of sertoli cell tumor tissue of dogs.

The correlation of lipoid to ketosteroid in interstitial cells was first emphasized by Pollack (1942) with Bennett's phenylhydrazine reaction (1940) for carbonyl groups in tissue. Firminger (1950) demonstrated androgenic steroid in interstitial cells by ultraviolet absorption spectromicroscopy.

The present study demonstrates (1) that ketosteroid is present in some sertoli cells as well as in interstitial cells, (2) that the ketosteroid in interstitial cells is for the most part alkali insoluble (androgens) and in sertoli cells alkali soluble (estrone), (3) that in tumors arising from these cells, the type of ketosteroid corresponds to the type of cell, and (4) that the interstitial cells may in some cases also contain a small amount of estrone. The method used in this study gives no information concerning the distribution of non-ketonic steroids (estradiol, pregnandiol) which may be present in these tissues.

SUMMARY

The testes of various species of animals were examined with a method for the histochemical demonstration of active carbonyl groups. Since it was shown that the carbonyl groups were ketonic in nature and occurred in lipoid material in tissue known to contain steroid hormones, it is presumed though not conclusively proved that ketosteroids were responsible for the reaction. Included are a series of interstitial and sertoli cell tumors of dogs, three experimentally produced interstitial cell tumors of mice, a group of human testes at various ages and six human seminomas. From this study the following may be concluded:

1. Germinal epithelium in all species, and seminomas, which are thought to arise from germinal epithelium, contained neither lipoid nor ketosteroid. This was also the finding in germinal epithelium, interstitial cells and sertoli cells of human embryos and children under 10 years of age.

2. Interstitial cells in most of the species contained varying quantities of ketosteroid. Tumors arising from interstitial cells, including those arising

FIG. 19. Experimental interstitial cell tumor of mouse (226). The cytoplasm of tumor cells contains granular diffusely distributed ketosteroids. Some areas of the tumor (lower right) contained little ketosteroid. Nuclei are unstained. Ketosteroid reaction. ×180.

FIG. 20. Same section as fig. 19. The granular distribution of the ketosteroid in the cytoplasm of tumor cells is evident. The nuclei are unstained. Ketosteroid reaction. ×1350.

spontaneously in the dog, and those produced experimentally in the mouse, generally contained large quantities of ketosteroid.

3. Sertoli cells varied considerably in their content of ketosteroid in different species. Tumors deriving from sertoli cells in the dog contained varying quantities of ketosteroid, usually less than that found in interstitial cell tumors.

4. In the human adult, both lipoid and ketosteroid were demonstrated in interstitial and sertoli cells.

5. Because of the alkali solubility of estrone in contrast to the androgenic ketosteroids, it was possible to demonstrate that the ketosteroid of sertoli cell tumors was mostly estrone and to a less extent in the case of normal sertoli cells. The ketosteroids of interstitial cell tumors were mostly androgens, but normal interstitial cells had variable amounts of estrone by this technique.

Acknowledgment

We are indebted to Drs. Monroe Schlesinger, Leopold Reiner, Sidney Farber, Shields Warren, Benjamin Castleman, and G. Boggs, Harvard Medical School, and to Dr. Stanley Robbins, Boston University and Tufts Medical School for human testicular material, to Dr. C. A. Pfeifer, Yale Medical School, Dr. R. E. Scully and Dr. D. Coffin, Harvard Medical School for the testicular tumors of the dog, and to Dr. W. U. Gardner, Yale Medical School for the experimentally induced testicular tumors of the mouse.

Acknowledgment for technical assistance is due Miss Barbara Fine and Mr. Myron Milden.

REFERENCES

ARON, M.: *Memoires de la Soc. de Biol.* **85:** 482. 1921.
ARON, M.: *Archives de Biol.* **34:** 1. 1925.
ARON, E.: *C. R. Soc. de Biol.* **102:** 1062. 1929.
ASHBEL, R. AND A. M. SELIGMAN: *Endocrinology* **44:** 565. 1949.
BENNETT, S. A.: *Am. J. of Anat.* **67:** 151. 1940.
BENOIT, J.: *C. R. de la Soc. de Biol.* **87:** 1382. 1925.
BOUIN, P. ET P. ANCEL: *Arch. de Zool Exper. et Gen.* **1:** Ser 4. 437. 1903.
BOUIN, P. ET P. ANCEL: *Memoires de la Soc. de Biol.* **55:** 1397. 1903.
BOUIN, P. ET P. ANCEL: *Memoires de la Societe de Biol.* **56:** 84. 1904.
BOUIN, P. ET P. ANCEL: *Memoires de la Societe de Biol.* **56:** 97 1904.
BOUIN, P. ET P. ANCEL: *Memoires de la Societe de Biol.* **56:** 335. 1904.
CHAMPY, C.: *Memoires de la Societe de Biol.* **64:** 895. 1908.
FIRMINGER, R.: *Proc. Histochem. Soc. in J. Nat. Cancer Instit.* **10:** 1350. 1950.
GARDNER, W. U.: *Cancer Research* **3:** 92. 1943.
GARDNER, W. U.: *Cancer Research* **5:** 497. 1945.
GARDNER, W. U.: *Cancer Research* **8:** 397. 1948.
HOOKER, C. W. AND C. A. PFEIFFER: *Cancer Research* **2:** 759. 1942.
HUGGINS, C. AND P. V. MOULDER: *Cancer Research* **5:** 510. 1945.
HUMPHREY, R.: *Am. J. of Anat.* **29:** 213. 1921.
LEYDIG, F.: *Ztsch. F. Wiss. Zool.* **2:** 1. 1850.

POLLACK, W. F.: *Anat. Record* **84:** 21. 1942.
ROMEIS, B.: *Kl. Woch.* **12:** 1640. 1933.
SELIGMAN, A. M. AND R. ASHBEL: *Bull. New Eng. Med. Center* **11:** 85. 1949.
SELIGMAN, A. M., O. M. FRIEDMAN AND J. E. HERZ: *Endocrinology* **44:** 584. 1949.
SELIGMAN, A. M. AND R. ASHBEL: *Cancer,* **4:** 579. 1951.
SELIGMAN, A. M. AND R. ASHBEL: *Endocrinology* in press.
SELIGMAN, A. M. AND R. ASHBEL: In preparation.
SERTOLI, E.: *Il Morgani Anno Settino.* **31:** 1865.
TEILUM, G.: *J. Clin. Endoc.* **9:** 301. 1949.
TEILUM, G.: *Acta Endocrinologica* **4:** 43. 1950.
WHITEHEAD, R. H.: *Am. J. of Anat.* **3:** 167. 1904.
WISLOCKI, G. B.: *Endocrinology* **44:** 167. 1949.

NOTES AND COMMENTS

FUNCTION OF THE ADRENAL GLAND IN SPERM EJACULATION BY MALE FROGS

The possibility exists of a relationship between the adrenal gland and sperm ejaculation in frogs, since it has been reported by Robbins and Parker (1949) and confirmed by H. and M. Hinglais (1949) that *Xenopus laevis* and *Rana pipiens* react to the injection of epinephrine with sperm ejaculation. *Xenopus laevis* was reactive to epinephrine, benzedrine and ephedrine throughout the year, while *Rana pipiens* was reactive to epinephrine only, and passed through a refractory period during the summer months of June, July and August. It has further been reported that *Bufo fowleri* and *Bufo arenarum* Hensel (Galli-Mainini, 1948) failed to respond to epinephrine.

The mechanism of the "epinephrine-ejaculation" is not clear, and it may be either a direct reaction or a chain reaction mediated by other organs. Mediation by the pituitary gland is improbable, since hypophysectomy does not interfere with the ejaculation test as shown either for epinephrine by Robbins and Parker (1949) or for gonadotropin by R. Black-Sandler (1950). Hence, the reverse chain reaction remains as a possibility viz. that gonadotropin provokes production of epinephrine and thus ejaculation. However, it is most probable that there exists no connection at all between "epinephrine-ejaculation" and "gonadotropin-ejaculation." The latter assumption is the most appealing since so far only 2 frog species have been shown to give "epinephrine-ejaculation," whereas all species give the "gonadotropin-ejaculation."

To clarify the hormonal mechanism underlying these reactions, we have studied the relationship between the adrenal gland and ejaculation in frogs.

We have tested the following three species available in Israel: *Rana pipiens*, *Hyla arborea*, and *Bufo viridis*. Our experiments were carried out between September and February, and 20 animals of each species were used. The frogs were injected into the dorsal lymph sack, and sperm was taken at hourly intervals for 8 hours after the injections. Details of the technique have been reported by Sulman and Sulman (1949, 1950).

1) *Injection of Adrenal Extracts*

A. *Adrenal Medulla*

The doses of epinephrine injected ranged from 0.1 gamma to 1 mg. for Rana and Bufo which both weighed 25 to 50 g.; for Hyla, which weighed only 5 to 10 g., the maximum dose employed was 100 gamma, since higher doses were toxic. We used the following preparations: L-epinephrine hydrochloride, L-epinephrine bitartrate (with ascorbic acid) and D,L-Arterenol HCl[1]).

The reaction with all 3 preparations was similar. The only species which showed sperm ejaculation was Rana. Hyla showed a typical hyperemia reaction of the whole ventral skin following doses of 20 to 100 gamma. All three species, however, developed a marked anuria during a 3 hour period after injection.

Received for publication May 9, 1951.

[1] We are very much indebted to Dr. M. L. Tainter of Winthrop-Stearns Inc., New York, who generously supplied the L-epinephrine bitartrate and the D,L-Arterenol.

B. *Adrenal Cortex*

The injection of adrenal cortex[2] extracts in Rana elicited minor ejaculation reactions which were apparently due to slight contaminations of epinephrine contained in these extracts. The same extracts also exerted the epinephrine effect in Hyla, namely hyperemia of the ventral skin. Injections of desoxycorticosterone[3] produced no significant effect (± reactions).

2. *Ablation of the Adrenals*

The adrenal gland is a small yellow thread of tissue which contains cortical and medullar cells, lying on both sides of the ventral part of the kidneys very near to the large vessels of these organs, but more than 3 mm. from the vas deferens and the testis which are situated at the cranio-ventral apex of the kidneys. It appears from this description that cortical adrenalectomy in frogs is best carried out by cauterization with a very fine thermocauter needle carefully guided by a binocular lens. It was done without narcosis to avoid any after-effect, since frogs thoroughly adrenalectomized by this technique die within 24 hours. Because of the proximity of the sperm-producing system and the lethal effect of the adrenalectomy we always employed controls with a sham operation which was carried out by thermocautery of the immediate lateral and medial vicinity (kidney tissue) of the adrenal cortex.

Adrenalectomy was performed in the morning, and was preceded one hour earlier by the injection of adrenal cortex extracts. The following cortical extracts were used: desoxy-corticosterone acetate[3] in doses of 1 gamma to 1 mg.; purified adrenal cortex extract from animal glands[2] in doses of 1 to 10 dog units; or the equivalent of 10–100 gamma cortisone; crude normal saline extract of whole mouse adrenal glands, prepared in our laboratory, in doses equivalent to $\frac{1}{4}$–1 mouse adrenal gland per frog. Two to five hours after the operation the frogs were injected with 10 to 100 I.U. chorionic gonadotropin[4] each, and 8 sperm samples were taken at hourly intervals.

Ejaculation was obtained by gonadotropin injections in adrenalectomized Rana, Bufo and Hyla, no matter whether they were treated previously with adrenal cortex extracts or not. This reaction did not occur, however, in frogs which died within 6 hours after the operation. In the latter cases the animals were rather weak at the time of gonadotropin injection and their reactions could not be adequately evaluated.

It is concluded that ejaculation in Rana is not associated with integrity of the adrenal cortex. The same holds, of course, for Bufo and Hyla where a connection between the gonadotropin effect and epinephrine does not exist prima facie.

SUMMARY

1. Epinephrine, which elicits ejaculation in Rana, had no such effect in Bufo and Hyla. However, it induced a marked hyperemia of the ventral skin in Hyla. The same holds true for Arterenol.

2. Adrenal cortex extracts gave similar results due to traces of epinephrine contained in them. Desoxycorticosterone acetate, however, was not active.

3. Adrenalectomy in Rana, Hyla and Bufo did not interfere with the ejaculation elicited by chorionic gonadotropin.

[2] Adrenal cortex extract was generously supplied by Dr. M. H. Kuizenga of Upjohn Co., Kalamazoo, Mich., and Eschatin by Mr. J. D. Heywood of Parke-Davis, Detroit.

[3] Doca was generously supplied by Dr. H. de Jager, of N. V. Organon, Oss, Holland.

[4] A.P.L. chorionic gonadotropin was most generously supplied by Dr. S. H. Jaros of Ayers, McKenna & Harrison, New York.

4. It is concluded that no hormonal relationship exists between "epinephrine-ejaculation" and "gonadotropin-ejaculation."

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REFERENCES

BLACK-SANDLER, R.: Thesis Hebrew University 1950.
GALLI-MAININI, C.: *J.A.M.A.* **138:** 121. 1948.
HINGLAIS, H. AND M. HINGLAIS: *Cpt. rend. Soc. biol.* **143:** 811. 1949.
ROBBINS, S. L. AND F. PARKER, JR.: *Endocrinology* **44:** 384. 1949.
SULMAN, F. G. AND E. SULMAN: *Harefuah* (Tel Aviv) **37:** 13. 1949.
SULMAN, F. G. AND E. SULMAN: *J. Obst. & Gyn.* (Brit. Emp.) **56:** 1014. 1949.
SULMAN, F. G. AND E. SULMAN: *J. Clin. End.* **10:** 933. 1950.

THE ACTION OF CORTISONE AND ACTH ON THYROID FUNCTION

It has been shown by means of radio active iodine uptake studies and protein bound iodine determinations that a depression of thyroid function is produced in animals and man following treatment with cortisone or ACTH (Hill *et al.*, 1950; Money *et al.*, 1950; Hardy *et al.*, 1950; Wolfson *et al.*, 1950), or in situations associated with high levels of circulating adrenal steroids such as follow stress or epinephrine injections (Williams *et al.*, 1949; Soffer *et al.*, 1949; Paschkis *et al.*, 1950). It has been suggested (Hill *et al.*, 1950) that this depression of function is due to an inhibition of pituitary thyrotrophin formation or release; this belief being based on the fact that the depression of thyroid uptake of I^{131} which follows ACTH or cortisone therapy can be counteracted by simultaneous administration of exogenous thyrotrophin. This does not, however, preclude the possibility that adrenal steroids may have some other inhibitory effect on the thyroid independent of thyrotrophin.

The purpose of this paper is to confirm the previously reported inhibitory effect of adrenal steroids and ACTH on the ability of the thyroid to take up iodine and to present evidence that this inhibition is not due to an interference with thyrotrophin production or secretion.

METHODS

Four month old male albino rats weighing approximately 200 gm. were used as experimental animals. These animals had been maintained since weaning on a diet low in iodine (400 μgm./Kgm.) but non-goitrogenic.

Each animal was given a 15 microcurie dose of carrier free radio-active iodine I^{131} intraperitoneally. Twenty-four hours were allowed to elapse before the first measurements of thyroid radio-iodine were made. Thyroid uptakes were measured in vitro by removal of the glands, hydrolysing them in 2% NaOH following which they were made up to a known volume with 2% NaOH and suitable aliquots taken for counting and compared with a standard prepared and counted under similar conditions. The distribution of radio-active thyroxine and non-thyroxine thyroid iodine was determined on the pooled hydrolysates of five glands following the procedure of Perlman, Morton and Chaikoff (Perlman *et al.*, 1941).

In vivo determinations of thyroid radio-iodine were made at 24 hour intervals for three to six days; the rate of decline of radio activity being considered an index of the rate of thyroid hormone secretion (Perry, 1951).

Received for publication May 9, 1951.

Cortisone acetate (Cortone, Merck) was administered in daily doses of 5 mgm. per rat 2.5 mgm. given subcutaneously and 2.5 mgm. intraperitoneally. ACTH (Armour) was administered in doses of 4 mgm. per rat subcutaneously twice daily. l-thyroxine was given in daily subcutaneous doses of 1.5 μgm. per animal.

RESULTS

In Table 1 is shown the effect of seven days treatment with ACTH and cortisone on the 24 hour uptake of radio-iodine. Also shown are the weights of the thyroid and adrenals following such treatment. It will be seen that both ACTH and cortisone depressed the uptake of iodine by the thyroids. Table 2 shows similar results on another group of cortisone treated rats. In this experiment the thyroids were pooled and the distribu-

TABLE 1. THE EFFECT OF ACTH AND CORTISONE ON THE THYROID UPTAKE OF RADIO-IODINE

	Weight gm.	Thyroid weight mgm.	Adrenal weight mgm.	24 hr. thyroid uptake of I^{131} % dose	
				per gland	per 10 mgm./gland
Controls	210	19.2	28.6	23.4	11.7
	199	10.0	25.8	13.9	13.9
	222	14.2	24.2	19.8	13.9
	215	17.4	24.8	16.5	9.5
	188	10.4	24.4	17.7	17.0
Mean	207	14.2	25.6	18.3	13.2
ACTH	178	10.6	24.8	13.7	12.9
	226	19.2	38.6	13.9	7.3
	222	13.0	33.0	13.3	10.2
	167	15.4	29.0	8.4	5.5
	237	16.2	33.2	15.2	9.4
Mean	202	14.9	31.6	12.9	9.0
Cortisone	217	16.0	23.6	10.8	6.8
	186	12.6	27.0	8.3	6.6
	213	16.6	22.4	14.4	8.7
	204	13.2	25.8	13.9	10.5
	178	12.6	20.0	16.9	13.4
Mean	200	14.2	23.7	12.8	9.2

tion of the radio-iodine between thyroxine and non-thyroxine iodine determined. In the cortisone treated animals there was less radio-iodine in both the thyroxine and non-thyroxine fractions than in those of the controls suggesting the inhibitory action of cortisone affects all stages in the uptake and synthesis of thyroid hormone.

The adrenal glands after ACTH and cortisone therapy showed evidence of the expected hypertrophy and atrophy respectively, as judged by the weights of these organs when compared with those of the control animals. On the basis of weight neither ACTH or cortisone had any effect on the thyroid gland.

The effect of ACTH and cortisone on the rate of release of radio-iodine from the thyroid gland is shown in Table 3. In this experiment treatment was started on the day of the initial in vivo counts, i.e. 24 hours after the administration of the tracer iodine. It will be seen that high levels of adrenal steroids did not have any effect on the rate of radio-iodine release.

TABLE 2. THE DISTRIBUTION OF RADIO-IODINE IN THE THYROID

	No. of Animals	Thyroid wt. mgm. ±s.d.	Adrenal wt. mgm. ±s.d.	24 hour uptake of I^{131} % dose			% Total Thyroid I^{131}	
				per gland	Thyroxine	Non-thyroxine	Thyroxine	Non-thyroxine
Controls	5	15.2 ±2.1	26.0 ±4.1	22.0	8.9	12.0	40	55
Cortisone	5	14.0 ±1.7	19.1 ±4.2	17.2	7.1	9.3	41	54

It seemed advisable to extend this latter experiment and observe the effect on the rate of hormone secretion following several days *pre* treatment with cortical hormone. At the same time the effect of cortisone was compared to that of thyroxine, an agent known to depress thyroid function by means of inhibition of pituitary thyrotrophin. Fig. 1 shows the results on three groups of rats: five control animals, a group of five animals treated for six days with 5 mgm. per day of cortisone and a third group of five animals treated for six days with 1.5 μgm. of thyroxine daily. The radio-iodine was given on the sixth day; in vivo counts were made 24 hours later and at 24 hour intervals for the next three days, the cortisone and thyroxine medication being continued during this time.

It will be seen that both thyroxine and cortisone depressed the amount of iodine taken up by the thyroid but whereas thyroxine produced a slower rate of release of the iodine taken up, the rate of release of hormone from the glands of the cortisone treated rats was similar to that of the control animals.

DISCUSSION

In agreement with previous workers (Hill *et al.*, 1950; Money *et al.*, 1950) it has been

TABLE 3. THE EFFECT OF ACTH AND CORTISONE ON THE RELEASE OF RADIO-IODINE FROM THE THYROID GLAND

	Weight gm.	Adrenal wt. mgm. at day 7	24 hour thyroid uptake I^{131} % dose	% of thyroid I^{131} in gland on day— (day 0 = 24 hrs. after I^{131})					
				0	1	2	3	5	6
Controls	230	26.0	17.6	100	60	43	30	14	10
	223	27.6	23.8	100	67	47	34	16	11
	219	35.6	20.0	100	72	58	46	25	21
	229	30.4	19.1	100	68	47	35	16	13
	241	33.4	25.6	100	53	31	21	8	7
Means	228	30.6	21.2	100	64	45	35	16	13
ACTH	204	29.6	23.6	100	66	47	36	16	11
	228	29.2	23.2	100	68	48	37	19	14
	202	48.2	21.6	100	69	50	36	16	13
	210	37.0	24.1	100	63	42	32	12	10
	208	40.0	21.0	100	69	52	38	18	14
Means	213	36.8	22.7	100	67	48	36	16	12
Cortisone	222	28.2	19.6	100	66	42	28	15	11
	200	17.0	24.0	100	63	39	27	12	9
	211	20.4	25.1	100	66	44	32	15	12
	215	—	18.7	100	63	—	—	—	—
	227	21.2	23.5	100	68	51	40	22	17
Means	219	21.7	22.2	100	65	44	34	16	12

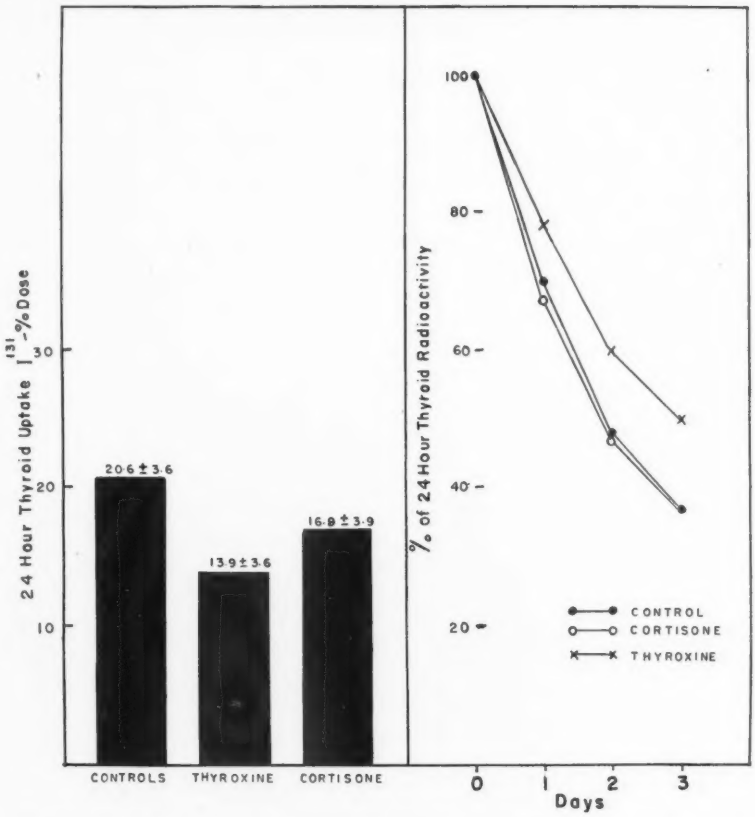

FIG. 1. The effect of Cortisone and Thyroxine on the uptake and release of Radio-Iodine by the thyroid gland.

shown that high levels of cortical hormones, either exogenous or endogenous interfere with the ability of the thyroid to take up iodine.

It has been postulated (Wolfson *et al.*, 1950; Hill *et al.*, 1950) that the decreased uptake of I^{131} is a consequence of pituitary thyrotrophin inhibition by the high levels of steroids. The results reported here suggest that this is not the mechanism. Thus a lowered production of thyrotrophin brought about by means of treatment with thyroxine led to both a decreased uptake and a decreased rate of release of radio-iodine from the gland whereas cortical hormone while interfering with the uptake of radio-iodine by the thyroid had no effect on its rate of release. Evidence for the concept that cortical hormones do not interfere with thyrotrophin production was provided by the absence of thyroid atrophy as judged on a weight basis after seven days treatment with ACTH and cortisone.

SUMMARY

1. ACTH and cortisone depressed the uptake of radio-iodine by the rat thyroid.

2. This depressed uptake was reflected in a lowering of both the thyroxine and non-thyroxine radio-iodine of the gland.

3. The rate of radio-iodine release by the thyroid gland was not affected by high levels of cortical hormones whereas it was lowered by thyroxine, an effect presumably mediated by thyrotrophin.

4. It is concluded that the depressed iodine uptake produced by adrenal cortical hormones is not due to an inhibition of pituitary thyrotrophin and it is suggested that the action may be a direct one on the thyroid gland.

Acknowledgments

The National Research Council of Canada provided financial aid and the cortisone used in this work. The ACTH was supplied through the kindness of Dr. J. Mote, Armour and Company. Thanks are due to Miss Margaret Hammett for technical assistance and to Dr. J. Doupe for helpful criticism.

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REFERENCES

HARDY, J. D., CECILIA RIEGEL AND ELEANOR ERISMAN: *Am. J. Med. Sc.* **85:** 199. 1950.

HILL, S. R., R. S. REISS, P. H. FORSHAM AND G. W. THORN: *J. Clin. Endocrinol.* **10:** 1425. 1950.

MONEY, W. L., L. KIRSCHNER, L. KRAINTZ, P. MERRILL AND R. W. RAWSON: *J. Clin. Endocrinol.* **10:** 1282. 1950.

PASCHKIS, K. E., A. CANTAROW, T. EBERHARD AND D. BOYLE: *Proc. Soc. Exper. Biol. and Med.* **73:** 116. 1950.

PERLMAN, I., M. E. MORTON AND I. L. CHAIKOFF: *J. Biol. Chem.* **139:** 449. 1941.

PERRY, W. F.: *Endocrinology* **48:** 643. 1951.

SOFFER, L. J., J. L. GABRILOVE AND J. W. JAILER: *Proc. Soc. Exper. Biol. and Med.* **71:** 117. 1949.

WILLIAMS, R. H., H. JAFFE AND C. KEMP: *Am. J. Physiol.* **159:** 291. 1949.

WOLFSON, W. G., W. H. BEIERWALTES, W. D. ROBINSON, J. F. DUFF, J. R. JONES, C. T. KNORPP AND E. MIOKO: *J. Lab. Clin. Med.* **36:** 1005. 1950.